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EFFECTS OF SUSPENDED MUSSEL CULTURE ON THE MACROZOOBENTHOS IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA, ITALY)

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ABSTRACT

This paper presents the results of a study of benthic community and sediments beneath a mussel culture and at a control site. The study was conducted on a monthly basis from May 1998 to April 1999 in the Gulf of Trieste (northern Adriatic Sea). The two sites had comparable sediment texture, while organic carbon and nitrogen content was higher beneath the mussel farm. Number of species, abundance and diversity indexes were similar at the two sites, while the biomass was, on average, twice as large beneath the farm. In general, the impact on the benthos did not appear to be very strong.

Key words: mussel cultures, macrozoobenthos, disturbance assessment, Adriatic Sea

EFFETTI DELLE MITILICOLTURE SUL MACROZOOBENTHOS DEL GOLFO DI TRIESTE (ALTO ADRIATICO, ITALIA)

SINTESI

L'impatto sul macrozoobenthos delle mitilicolture nel Golfo di Trieste è stato valutato analizzando le modificazioni della struttura della comunità macrobentonica ed i parametri abiotici maggiormente significativi. I campionamenti sono stati eseguiti a cadenza mensile, tra maggio 1998 ed aprile 1999, sotto ad una mitilicoltura ed in una stazione di riferimento. Le due stazioni hanno la stessa tessitura (30% silt, 70% argilla), mentre le percentuali di carbonio organico e di azoto totale sono, rispettivamente, il 30% e 25% più elevate sotto la mitilicoltura. Il numero di specie, l'abbondanza e gli indici di diversità presentano valori simili, mentre la biomassa è il doppio sotto la mitilicoltura. La classificazione e l'ordinamento nMDS (non-metric multidimensional scaling) separano chiaramente le due stazioni, inoltre le curve ABC (Comparazione Abbondanza-Biomassa) evidenziano un disturbo leggero sotto la mitilicoltura. Il debole impatto sul macrozoobenthos può essere dovuto alla riduzione, negli ultimi anni, della produzione della mitilicoltura in esame, ma soprattutto, alle caratteristiche idrodinamiche dell'area che sembrano avere un ruolo importante nella dispersione dei biodepositi.

Parole chiave: mitilicolture, macrozoobenthos, valutazione del disturbo, Mar Adriatico

INTRODUCTION

The farming of the blue mussel *Mytilus galloprovincialis* on hanging longlines is the main mariculture activity in the Gulf of Trieste, where it has been carried out traditionally for several hundred years and industrially for more than 40 years. Mussel farms nowadays cover an area 15 km long and 100 m wide, along the NE coast of the Gulf. At the end of the 1980's, about 8,500 tons year^{-1} was produced. Then, toxic algal blooms and occasional mucus aggregates during the summer, coupled with a general crisis of the fisheries activity, caused a drastic production fall reaching 2,000 tons in 1996 (Franzosini, 1998). Recovery has begun since, and production reached 4,000 tons year^{-1} in 2000 (Orel & Zamboni, 2004). In spite of the slow recovery, mussel farms currently represent a very important economic asset for the local fishery industry.

Mussel cultures produce large amounts of biodeposits (faeces and pseudofaeces) that cause increased deposition of organic material in the benthos beneath them (Kautsky & Evans, 1987; Hatcher *et al.*, 1994). Dahlbäck & Gunnarsson (1981) estimated the sedimentation rate of organic matter under a culture to be up to three times higher than at a control site, involving carbon and nitrogen accumulation in the sediment (Hall *et al.*, 1990, 1992). The enrichment in organic matter causes changes in the sediment composition (Mirto *et al.*, 2000) as well as an increase in oxygen consumption, causing bottom hypoxia or anoxia (Mattson & Lindén, 1983). In turn, these conditions influence the structure of the macrobenthic communities (Brizzi *et al.*, 1995; Chamberlain *et al.*, 2001), leading to the dominance of opportunistic polychaetes and to macrofaunal biomass drops (Hargrave *et al.*, 1993). The smaller benthic components, such as meiofaunal and microbial communities, are deeply influenced too (Mirto *et al.*, 2000; La Rosa *et al.*, 2001). The impact can vary considerably among locations, both in extent and degree, depending on the environmental characteristics of the area (Grant *et al.*, 1995; Svane & Setyobudiandi, 1996).

The aim of this study was to evaluate the effects of suspended mussel cultures on the underlying macrobenthic fauna, by analysing the changes in the community structure during one year. The most significant features of the sediment (redox potential, organic carbon and nitrogen) were also analysed to assess the differences between the area underneath the cultures and a control site.

MATERIALS AND METHODS

The Gulf of Trieste is a shallow bay (maximum depth 26 m), characterized by wide temperature changes between winter and summer (e.g. from surface layer minimum of about 7 °C to maximum ≥26 °C). Thermal stratification in the summertime and winter mixing, as well as high sedimentation rates of river sediment outputs are also typical of the area.

Sampling was performed monthly from May 1998 to April 1999 at a mussel farm located along the NE coast of the Gulf (Fig. 1). This farm is operative since 1976, with an annual production of about 100 tons, with a reduction of about 40% in the years preceding the study, like at many farms of the Gulf. In this area the surface currents, as shown by Martinčić *et al.* (1998), range from 0 to 28 cm s^{-1} (mean value of 8 cm s^{-1}) due to the tidal influence, but first of all to the effect of the winds.

Two stations were analysed: 1) station M, located inside the farm, on muddy sediments (30% silt, 70% clay) almost completely covered by a "mussel-carpet", constituted of living and dead mussels falling from the ropes, and by plastic nets used as culture ropes, at a depth of 13 m; 2) station B, as the control site, located about 100 m away from the culture, not impacted by the mussel culture, with similar depth (14 m) and sediment texture (29% silt, 71% clay). Temperature (T) (°C), salinity (S) (psu) and dissolved oxygen (D.O.) (% saturation) were measured on vertical profiles using a CTD Idronaut mod. 401 probe.

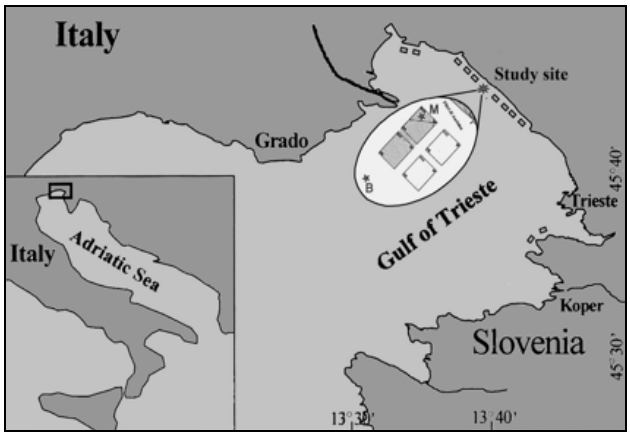

Fig. 1: Map of the study area and sampling stations.
Sl. 1: Zemljevid preučevanega območja in vzorčišč.

Three core samples for redox potential measurements, organic matter and sediment particle size analyses were collected every month by SCUBA-divers by hand-coring. The redox potential (Eh) of the sediment was immediately measured at 1.5 cm intervals down to 10.5 cm, using a redox probe, standardized in Zobell's solution, in inert medium (Standard Methods, 1992). The surface layers of the samples were freeze-dried, homogenized and sieved through a 300 µm mesh sieve, after acidification (Hedges & Stern, 1984) to remove the carbonates. Analyses of organic carbon and total nitrogen were performed on three replicates, using a Perkin Elmer 2400 CHNS/O analyzer calibrated with acetani-

lide at a combustion temperature of 1050 °C. The textural composition of the sediment samples was defined by dry sieving (sandy fraction) and by using the Micrometrics Sedigraph 5000 ET Particle Size Analyzer for silt and clay composition, following procedures reported by Covelli & Fontolan (1997).

At each station, three replicate samples were collected with a 0.1 m^2 van Veen grab and sieved on a 0.5 mm mesh sieve. The retained fauna was preserved in 4% buffered formaldehyde solution, sorted and identified to the highest possible taxonomical level. Biomass was determined as ash-free dry weight (AFDW). The species were grouped into five feeding guilds: carnivores/omnivores, suspension feeders, surface deposit feeders, sub-surface deposit feeders and herbivores (grazers) (Fauchald & Jumars, 1979; Bachelet, 1981).

Species richness, abundance, Shannon-Wiener diversity index ($\log_e$) and evenness index were used to analyse the community structure. The cluster analysis and the non-metric multidimensional scaling (MDS) were carried out on square root-transformed abundance data using the Bray-Curtis similarity measure. The SIMPER procedure was applied to determine the good discriminating species for dissimilarity between the affected area and the control station. To detect disturbances on the benthic community, the Abundance Biomass Comparison (ABC) method (Warwick, 1986) and W-statistics (Clarke & Warwick, 2001) were applied:

$$W = \sum_{i=1}^{S} (Bi - Ai) / [50(S-1)]$$

Bi and Ai are the biomass and abundance values for each rank (i) in an ABC curve, and S is the number of species. W takes values in the range from –1 (severely disturbed) to +1 (undisturbed), so the community should have a negative W value if it has been disturbed. Univariate and multivariate analyses were performed using PRIMER 5 software (Clarke & Warwick, 2001).

RESULTS

Abiotic factors

The two stations were characterized by very similar hydrological features. Annual mean temperatures at the bottom were slightly higher at station M (14.8±5.3 °C) than at station B (14.4±5.0 °C), ranging from a minimum of 8.1 °C to a maximum of 21.8 °C at the farm site, and from 7.9 °C to 21.1 °C at the control site. Salinity varied from 35.87 psu to 37.85 psu at the farm site, and from 35.87 psu to 37.88 psu at the control site. The surface layer showed low values at both stations in October and April, in coincidence with larger river inputs during the rainiest periods. Dissolved oxygen values at the bottom ranged from 70.07% to 101.22% at station M and from 73.54% to 101.86% at station B. The lowest values were registered at both stations in September.

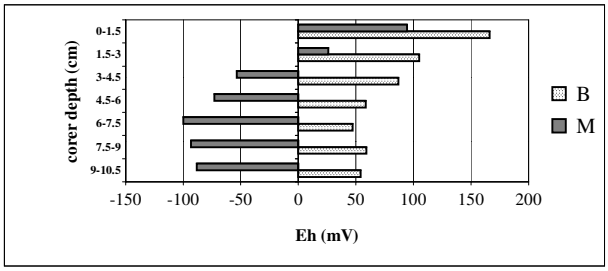

Fig. 2: Mean values of the redox potential of the sediment of the mussel farm (M) and of control station (B).
Sl. 2: Srednja vrednost redox potenciala v sedimentu pod gojiščen školjk (M) in na kontrolni postaji (B).

Eh mean values were higher at station B in all sediment levels (Fig. 2), but the monthly profiles were very variable. At the surface layer (0-1.5 cm), values ranged from –52 mV to 239 mV at station M, and from –37 mV to 422 mV at station B. Considering the mean values of Eh in the different layers, station M showed positive values down to 3 cm depth, while mean values at station B were always positive (Fig. 2).

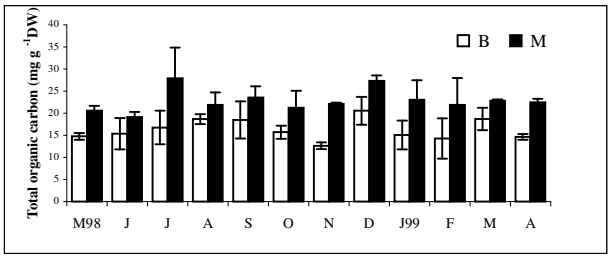

Fig. 3: Temporal variation of the total organic carbon.
Sl. 3: Časovno variiranje skupnega organskega ogljika.

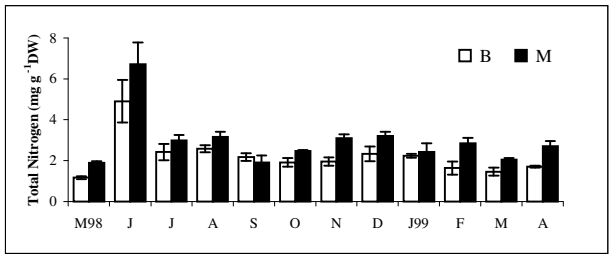

Fig. 4: Temporal variation of the total nitrogen.
Sl. 4: Časovno variiranje skupnega dušika.

Elemental analysis of the surface sediment showed organic carbon and total nitrogen values (as mg g^{-1} dry sediment) always higher beneath the mussel farm (Figs. 3, 4), but the t-test demonstrated significant differences between the two stations only for organic carbon ($p < 0.05$). The organic carbon content at station M ranged from 19.17 (±1.83) mg g^{-1} (June) to 27.83 (±7.02) mg g^{-1} (July). At station B, the highest value measured was 20.53 (±3.2) mg g^{-1} in December and the lowest 12.62 (±0.78) mg g^{-1} in November. The total nitrogen concentration was always higher at station M than at station B, except for September; values varied from 1.90 (±0.36) mg g^{-1} to 6.72 (±1.07) mg g^{-1} at station M, and from 1.16 (±0.07) mg g^{-1} to 4.91 (±1.05) mg g^{-1} at station B. The C/N ratios at both stations were generally lower than 12, with highest values in September at station M (14.47±3.01) and in March at station B (15.00±0.2), while the lowest values were found in June at both stations (Fig. 5).

Faunal composition

A total of 163 taxa were identified at station M and 170 at station B, among which 140 were mutual taxa between the two stations. Polychaetes were always the richest group, followed by molluscs, crustaceans and echinoderms (Tab. 1). The monthly pattern of the number of taxa was similar under the mussel farm and at the control site. In May we observed the highest number at both stations, with 85 species at station M and 108 at station B, whereas the lowest values were found in winter, with 41 taxa at station M (February) and 50 taxa at station B (March) respectively (Fig. 6).

The total abundance was not significantly different between the stations. At each site, the highest values were found in May, due to the dominance of two polychaetes: *Prionospio cirrifera* representing 58% of all individuals at station M, and *Lumbrineris gracilis* representing 23% at station B. Low values were recorded in winter (Fig. 7).

Tab. 1: Number of taxa in the main macrobenthic groups at the two stations.
Tab. 1: Število taksonov v glavnih makrobentoških skupinah na obeh postajah.

Number of taxa	Station M	Station B
Polychaetes	78	85
Molluscs	41	45
Crustaceans	23	19
Echinoderms	10	11
Others	11	10
Total	**163**	**170**

The analysis of abundance of the main taxonomic groups (polychaetes, molluscs, crustaceans, echinoderms) showed that polychaetes were always dominant at both stations (on average 85% at station B and 72% at station M) and in all samples. Crustaceans, molluscs and echinoderms were on average more abundant at station M than at station B, with 15%, 9%, 4% and 6%, 6%, 3% respectively.

The ten most abundant taxa at each station are listed in Table 2. Species composition at station M was dominated by the polychaetes *P. cirrifera* and *L. gracilis*. The former was very abundant only in May (3239 ind m^{-2}), whereas the latter was abundant every month. The dominant species at station B were the polychaetes *L. gracilis*, *Aricidea* spp. and *Maldane glebifex*. Some species were exclusively present at one station. *Haliotis lamellosa tuberculata* and *Paracentrotus lividus* were found only at station M, whereas three polychaete species, *M. glebifex*, *Lumbrineris tetraura* and *Sthenolepis yhleni*, were recorded only at station B. The mollusc *Nucula nucleus* and the crustaceans *Pisidia longimana* and *Athanas nitescens* were significantly more abundant at station M than B, on average 98 vs 20 ind m^{-2}, 108 vs 32 ind m^{-2} and 57 vs 8 ind m^{-2} respectively.

Tab. 2: Top ten ranked taxa, mean density and percentage at the two stations.
Tab. 2: Prvih deset najpomembnejših taksonov, njihova srednja gostota in deleži na obeh postajah.

Station M			Station B		
Taxa	**Ind. m^{-2}**	**%**	**Taxa**	**Ind. m^{-2}**	**%**
Prionospio cirrifera	350	17.84	*Lumbrineris gracilis*	492	25.61
Lumbrineris gracilis	195	9.95	*Aricidea* sp.p.	251	13.05
Aricidea sp.p.	168	8.56	*Maldane glebifex*	121	6.32
Pisidia longimana	108	5.48	*Eunice vittata*	103	5.37
Nucula nucleus	98	5.01	*Pomatoceros triqueter*	84	4.37
Pomatoceros triqueter	86	4.37	*Lumbrineris latreilli*	61	3.17
Eunice vittata	65	3.30	*Levinsenia gracilis*	57	2.95
Lumbrineris latreilli	61	3.13	Syllidae indet.	45	2.33
Athanas nitescens	57	2.89	*Prionospio cirrifera*	42	2.18
Gammaridae indet.	51	2.58	Gammaridae indet.	40	2.07
		63.10			67.41

The Shannon-Wiener diversity index and evenness index did not show significant differences between the stations. Shannon-Wiener average was 2.99 (±0.37) at station M and 3.04 (±0.24) at station B, and evenness average was 0.76 (±0.09) at station M and 0.74 (±0.05) at station B.

In order to evaluate the percentage of sensitive/tolerant species, the species found at the two sites were assigned to the ecological groups reported by Simboura & Zenetos (2002). Only half of the species identified could be assigned, therefore the number of sensitive/tolerant species was reported, without the percentage values. The results should be considered with care, as only half of the community is represented. At station M, 22 sensitive and 56 tolerant species were found, while at station B, 32 sensitive and 59 tolerant species were recorded.

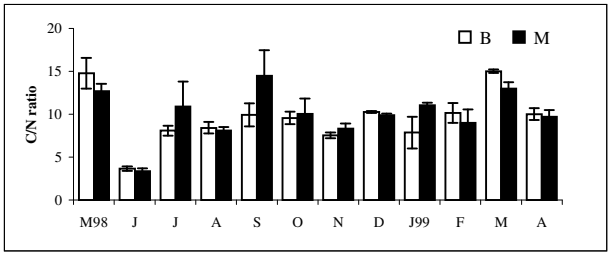

Fig. 5: Temporal variation of C/N ratio.
Sl. 5: Časovno variiranje C/N razmerja.

Biomass (not considering *M. galloprovincialis* fallen from the ropes) was higher beneath the mussel culture with a mean value of 41.65 (±30.73) g AFDW m^{-2} compared to a value of 22.73 (±15.20) g AFDW m^{-2} at the control site. At station M, the highest value was recorded in August (114.05 g AFDW m^{-2}) and the lowest in December (3.03 g AFDW m^{-2}) (Fig. 8), while at station B, the highest value of biomass was 60.57 g AFDW m^{-2} in December and the lowest in March (4.52 g AFDW m^{-2}). At both stations, on average 68% of the biomass was composed by epifauna, above all anthozoa and ascidiacea, and 32% of infaunal organisms.

Beneath the mussel farm, in addition to anthozoa and ascidiacea (Tab. 3), the most representative species, in terms of biomass, were the polychaete *Marphysa sanguinea* and the molluscs *Hexaplex trunculus* and *Lima exilis,* while at the control site the polychaetes *M. sanguinea* and *Chaetopterus variopedatus* were the most important.

Considering the feeding guilds, suspension feeders (anthozoa, ascidiacea, *L. exilis, Cucumaria planci)* dominated by biomass at station M, representing 51% of the total biomass (Tab. 4), while carnivores represented 34% in biomass, mostly due to the polychaete *M. sanguinea* and the mollusc *H. trunculus*. Similarly, at station B, suspension feeders dominated (68%), followed by carnivores (21%). Regarding the other trophic groups, grazers and surface deposit feeders were more important as biomass beneath the mussel farm, whereas surface deposit feeders and sub-surface deposit feeders played a more important role at the control site.

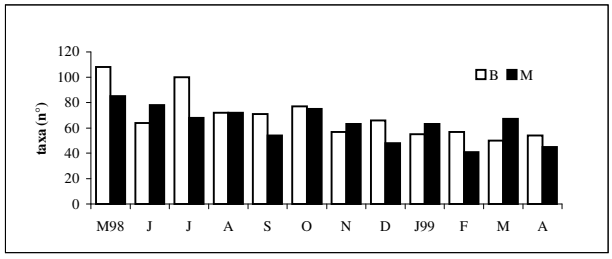

Fig. 6: Number of taxa of the macrobenthic fauna at station M and station B.
Sl. 6: Število taksonov makrobentoške favne na postaji M in postaji B.

Tab. 3: Biomass and relative percentage of the most representative species.
Tab. 3: Biomasa in deleži najbolj reprezenativnih vrst.

Station M			Station B		
Taxa	**g AFDW m^{-2}**	**(%)**	**Taxa**	**g AFDW m^{-2}**	**(%)**
Anthozoa indet.	6.75	16.2	Ascidiacea indet.	8.16	35.9
Marphysa sanguinea	5.60	13.4	Anthozoa indet.	3.78	16.6
Ascidiacea indet.	4.83	11.6	*Marphysa sanguinea*	2.38	10.5
Hexaplex trunculus	4.75	11.4	*Chaetopterus variopedatus*	1.30	5.7
Lima exilis	3.11	7.5	*Hexaplex trunculus*	0.94	4.1
Cucumaria planci	2.46	5.9	*Ophiothrix quinquemaculata*	0.58	2.5
Paracentrotus lividus	2.16	5.2	Nemertea indet.	0.55	2.4
Nucula nucleus	1.99	4.8	*Dasybranchus caducus*	0.51	2.2
		76			80

In term of abundance, surface deposit feeders (46%) dominated under the mussel farm due to some small-sized polychaetes, such as *Prionospio cirrifera* and *Aricidea* spp., followed by carnivores (39%). At station B, carnivores (47%) dominated, followed by surface deposit feeders (32%), while sub-surface deposit feeders were found almost exclusively at the control site.

Tab. 4: Feeding guilds: percentage of biomass and abundance. SF: Suspension feeders; C/O: Carnivores/Omnivores; SDF: Surface Deposit Feeders; SSDF: Sub-Surface Deposit Feeders; G: Grazers (herbivores).
Tab. 4: Prehranjevalni cehi: delež biomase in številčnosti. SF: suspenziofagi; C/O: karnivori/omnivori; SDF: površinski detritofagi; SSDF: podpovršinski detritofagi; G: rastlinojedci.

	Biomass (%)		Abundance (%)	
	Station M	**Station B**	**Station M**	**Station B**
SF	51.0	68.0	14.0	12.0
C/O	33.7	21.0	38.8	47.0
SDF	6.9	4.8	45.7	32.0
SSDF	0.1	3.7	0.6	8.7
G	8.3	2.5	0.9	0.3

Cluster analysis and MDS provided a clear separation between the two sites, with the only exception of the May sample under the farm, which is in the dendrogram included in the control site group (Figs. 9a, 9b). The ABC curves, generated from the combination of monthly data, indicate a slight disturbance in the mussel site (Fig. 10): the curves lie somewhat close together and cross each other. W values, calculated for each station and month, were positive for both stations, except for station M in May, which showed a slightly negative value (Tab. 5). The values ranged from –0.008 to 0.314 at station M, with a mean of 0.195 (±0.093); at station B, they ranged from 0.128 to 0.355, with a mean of 0.209 (±0.058).

Table 6 shows the results of breaking down the dissimilarities between the two sites into species contributions: the dissimilarity between the two sites was mainly due to *M. glebifex, N. nucleus* and *L. gracilis*, which were the species with higher Diss./SD ratio. Nearly 50% of the contribution to dissimilarity was accounted for by the first eight species listed.

Tab. 5: W-statistic values corresponding to the monthly ABC curves of the two stations.
Tab. 5: W – statistične vrednosti, ki ustrezajo mesečnim krivuljam ABC obeh postaj.

	station M	**station B**
M '98	-0.008	0.128
J	0.232	0.24
J	0.215	0.21
A	0.304	0.177
S	0.31	0.199
O	0.139	0.228
N	0.218	0.182
D	0.125	0.355
J99	0.15	0.247
F	0.314	0.193
M	0.176	0.145
A	0.162	0.209
Average	**0.195**	**0.209**
SD	0.093	0.058

Tab. 6: Breakdown of average dissimilarity between the two stations into contributions from each species.
Tab. 6: Razčlemba povprečne različnosti med dvema postajama na prispevke vsake vrste.

	B	**M**	**Av. diss**	**Diss/SD**	**Contrib %**	**Cum. %**
	Av. abund	**Av. abund**				
Lumbrineris gracilis	491.67	195.28	7.61	1.42	12.71	12.71
Prionospio cirrifera	41.94	350.28	5.70	0.48	9.51	22.22
Aricidea sp.p.	250.56	168.06	4.43	0.88	7.39	29.61
Maldane glebifex	121.39	1.39	3.34	1.92	5.57	35.18
Decapoda	32.78	109.72	2.64	1.11	4.40	39.58
Pisidia longimana	31.67	107.50	2.61	1.01	4.36	43.94
Pomatoceros triqueter	83.89	93.33	2.33	1.23	3.89	47.83
Nucula nucleus	20.28	98.33	2.20	1.69	3.67	51.50
Average dissimilarity between stations = 59.90						

DISCUSSION AND CONCLUSIONS

The abiotic factors (T, S, D.O.) measured in the water column were similar for both stations. The D.O. lowest values were registered in September, when D.O. usually decreases in most of the bottom layers of the Gulf of Trieste. This is due to the summer thermal stratification of the water column (5–6 m at the beginning of spring until about 15 m at the end of summer) (Cardin & Celio, 1997).

According to many authors (Kaspar *et al.*, 1985; Hatcher *et al.*, 1994; Stenton-Dozey *et al.*, 2001), there is an increase of organic carbon and nitrogen under mussel cultures: in our study, the organic carbon and the total nitrogen concentrations were 30% and 25% higher beneath the mussel farm than in the control site respectively. The C/N ratio showed average values ≤ 12, indicating the marine origin of the organic material in the sediments (Wassmann, 1984). The low C/N ratio values in June, at both stations, could indicate the prevalence of protein-rich organisms with high nitrogen content and/or microbial activity. High C/N values (May and March) may highlight the prevalence of degradation processes of organic nitrogen in the water-sediment interface and the presence of allochthonous organic matter (Faganeli *et al.*, 1988). In turn, the higher C/N ratio values under the harvesting area in July, September and January could indicate accumulation of refractory organic material, derived mainly from faeces and decaying mussels and foulers (Stenton-Dozey *et al.*, 2001).

At station M, anoxic conditions were observed in the sediment, but only in the layers underlying the first 3 cm. From scuba-diving direct observations, no bacterial mats or black muds were detected. The sediment beneath the mussel farm was almost completely covered by a "mussel-carpet", constituted of living and dead mussels falling from the ropes, and by plastic nets used as culture ropes. A large number of mussels fall down from the overlying ropes, particularly during the first stages of development or in summer, before their harvesting, when the byssus threads break. The mussels commonly survive on the seabed for a few months, although three-year-old specimens have occasionally been collected (Brizzi *et al.*, 1995). In this way, the mussel cultures transfer to the benthic system large amounts of organic matter available for predators and scavengers, as well as faeces and pseudofaeces for deposit feeders (Frankenberg & Smith, 1967; Tenore *et al.*, 1973; Stuart *et al.*, 1982; Rosenberg & Loo, 1983).

Polychaetes represented the dominant group at both stations, which was expected considering that they normally constitute about 80% of abundance on muddy sediments (Somaschini *et al.*, 1996).

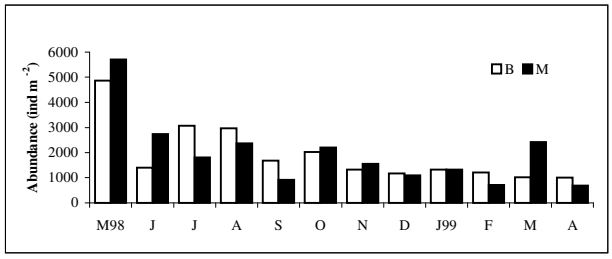

Fig. 7: Abundance of the macrobenthic fauna at station M and station B.
Sl. 7: Številčnost makrobentoške favne na postaji M in postaji B.

The analysis of the presence of sensitive and tolerant/opportunistic species highlighted at both stations a high number of tolerant species, which were the same as found commonly in all the muddy sediments of the Gulf of Trieste (Solis-Weiss *et al.*, 2001; Aleffi *et al.*, 2005). Moreover, it is to underline that on muddy bottoms (as found at the two stations) the fauna is generally dominated by tolerant species.

The percentage abundances of the main taxa were similar at both stations, except for the crustaceans that appeared to be more abundant under the mussel farm, especially the decapod *Pisidia longimana*. Another species of the same genus *Pisidia longicornis* has been found as dominant in the epifauna of the culture ropes in the Rìa de Arousa (Spain) (Román & Pérez, 1982) and as the main component in the diet of the predator crustaceans living there (González-Gurriarán *et al.*, 1995). According to Freire (1996), culture ropes could be considered an additional substratum for the development of epifaunal and macroalgal communities. In this case, the "mussel carpet" under the farm represents an ideal habitat and food supply for crustaceans and for some herbivores like the abalone *Haliotis lamellosa tuberculata* and the sea urchin *Paracentrotus lividus*, exclusively present at station M, but very frequent on the secondary hard-ground-community in the Gulf of Trieste (Zuschin & Pervesler, 1996).

The high abundance at station M of *Nucula nucleus*, a deposit feeder bivalve, should be noted, partly because it is one of the species that provided the greatest contribution to dissimilarity between affected area and control station. Another species of the same genus, *Nucula nitidosa,* was reported by Mattsson & Lindén (1983) as dominant at the start of mussel harvesting. *N. nucleus* is considered a species that spreads towards organic effluents (Elias, 1992). Grant *et al.* (1995) recorded another nuculoid, *Nuculana tenuisulcata,* as dominant under the ropes, due to an increased supply of sedimentary organic matter beneath the farm area.

The analyses of species richness, abundance and the Shannon-Wiener index did not reveal significant differences between the two sampling sites, and the diversity values were high compared to other areas of the Gulf of Trieste (Solis-Weiss *et al.*, 2001). Moreover, the high percentage (83%) of mutual species between the stations outlined the similarity also in species composition.

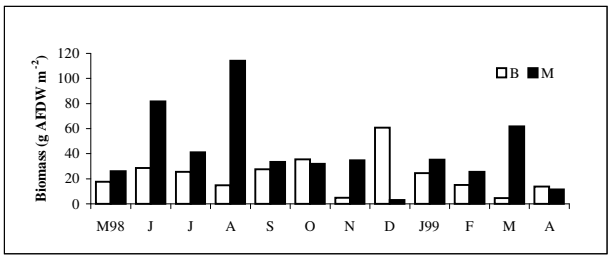

Fig. 8: Biomass of the macrobenthic fauna at station M and station B.
Sl. 8: Biomasa makrobentoške favne na postaji M in postaji B.

The biomass, on average, was twice as large at station M. This corresponds well with Hatcher *et al.* (1994) and Grant *et al.* (1995) results, since they have reported biomass values generally higher under mussel sites than at the control sites.

Suspension feeders and carnivores were dominant in biomass at both stations. However, in abundance, surface deposit feeders dominated under the farm and carnivores at the control site, in contrast to the study of Stenton-Dozey *et al.* (1999), who reported a dominance of deposit feeders, both in biomass and abundance, followed by carnivores beneath mussel cultures.

At each station, suspension feeders were represented by specimens with relatively high biomass, such as the ascidians (mostly *Microcosmus sp.*), the brittle star *Ophiothrix quinquemaculata* and, almost exclusively at station M, the holothurian *Cucumaria planci* and the bivalve *Lima exilis*. These species belong to one of the most widespread epibenthic communities in the Gulf of Trieste, known as *Ophiothrix-Reniera-Microcosmus* community (O-R-M) after the name of the three dominant genera (Fedra *et al.*, 1976). The O-R-M community is characterised by mobile and sessile suspension feeders, which are aggregates in the form of multi-species clumps (Stachowitsch & Fuchs, 1995). Ott & Fedra (1977) and Ott (1981) assumed that this community plays an important role in stabilizing the entire ecosystem by removing suspended material from the water column and storing it in the form of benthic biomass. Beneath mussel cultures, this epifaunal community had a higher biomass, because the "mussel carpet" and the plastic ropes lying on the bottom form an ideal substratum for attachment.

Carnivores, both predators and scavengers, were generally attracted by mussels falling from the culture ropes, as already noted in many studies (Tenore *et al.*, 1982; Kaspar *et al.*, 1985; Grant *et al.*, 1995; Crawford *et al.*, 2003) including Brizzi *et al.* (1995) in the Gulf of Trieste.

Remarkably, sub-surface deposit feeders were present exclusively at the control site, and mainly represented by the polychaete *Maldane glebifex*. This species highly contributed to the dissimilarity between the two stations: it was almost absent beneath the mussel farm, as already found under other mussel cultures in the Gulf of Trieste, probably because the mussel biodeposition alters the compactness of the sediment, preventing the building of its tube (Brizzi *et al.*, 1995). Moreover, the negative mean Eh values of the sediment underlying 3 cm at the station M could have contributed to the small percentage of sub-surface deposit feeders.

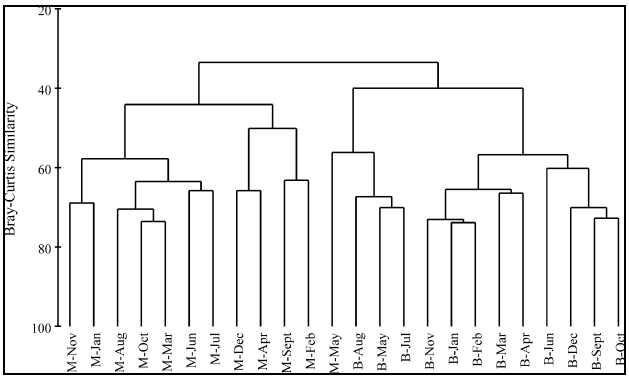

Fig. 9a: Dendrogram of hierarchical clustering of the monthly samples at the two stations.
Sl. 9a: Dendrogram hierahičnega grozdičenja mesečnih vzorcev na obeh postajah.

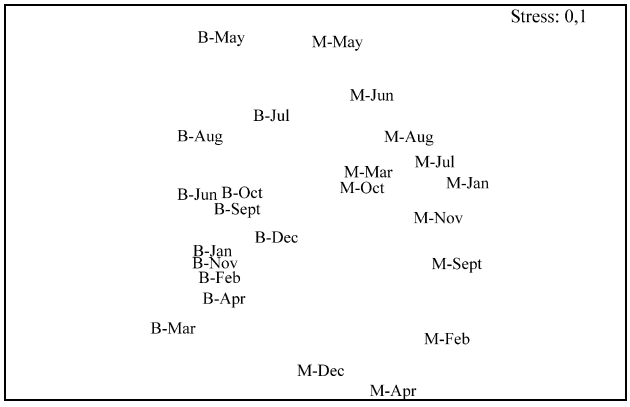

Fig. 9b: MDS plot for macrobenthic abundance at the two stations.
Sl. 9b: Večdimenzionalno skaliranje (MDS) številčnosti makrobentosa na obeh postajah.

Both cluster analysis and MDS provided a clear separation between the sites, likely due to the above-mentioned differences of species composition and abundance. The ABC method indicated a very slight impact of the mussel culture on the benthos, because the effects of mussel biodeposition did not drive the community to an early succession stage, to which the ABC method is more sensitive. Considering the W values, calculated monthly, only one sample (May-station M) showed a slightly negative value probably due to the higher presence and abundance of species with small body size.

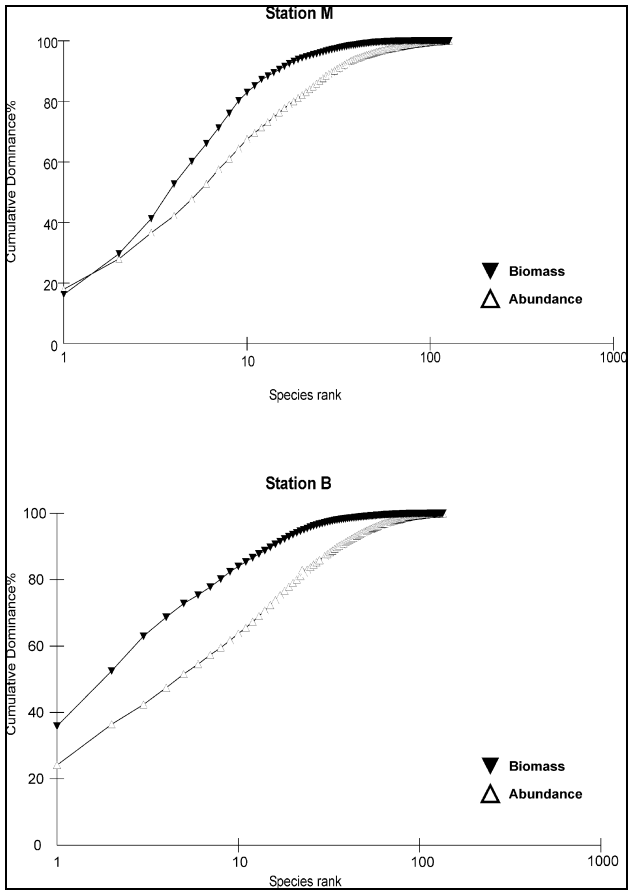

Fig. 10: ABC plots based on the monthly data for the two stations.
Sl. 10: ABC diagrama na osnovi mesečnih podatkov za obe postaji.

The presence of suspended cultures have induced some modifications on the bottom sediments, but as discussed in other studies (Baudinet *et al.*, 1990; Grant *et al.*, 1995; Crawford *et al.*, 2003; McKinnon *et al.*, 2003), the macrobenthic community does not appear to be under intense stress. The typical conditions of the communities exposed to a strong organic enrichment, as described by Pearson & Rosenberg (1978), are not present. The limited impact detected could be due to the decrease of production of this mussel farm in the previous years, but above all, to the presence, in the study area, of currents with mean values of 8 cm s^{-1} (maximum value 28 cm s^{-1}) (Martinčić *et al.*, 1998). Hydrodynamics sustained an efficient water circulation and, consequently, a limited biodeposition on the bottom. Anyway, the macrobenthic communities show some differences in biomass and species composition between the two sites. The degree and extent of the effects have been related, in many studies, to different factors such as the age of the farm, the densities of the organisms on the ropes or the hydrological characteristic of the area (Chamberlain *et al.*, 2001). In our study, hydrodynamics seem to play an important role in the dispersion of the biodeposits and in the consequent low level of impact found.

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UČINKI GOJENJA KLAPAVIC NA MAKROZOOBENTOS V TRŽAŠKEM ZALIVU (SEVERNI JADRAN, ITALIJA)

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POVZETEK

Avtorji prispevka so preučevali učinke gojenja školjk na makrozoobentos v Tržaškem zalivu (severni Jadran), in sicer z analiziranjem sprememb v strukturi združbe in ob upoštevanju najpomembnejših abiotskih parametrov. Študija je potekala od maja 1998 do aprila 1999 z mesečno frekvenco pod gojiščem klapavic in na kontrolni postaji kakih 100 m od gojišča. Tekstura morskega dna na preučevanih lokalitetah je bila enaka (30% mulja, 70% ilovice), medtem ko so bile koncentracije organskega ogljika in dušika za 30% oz. 25% večje pod gojiščem školjk. Po številu vrst in diverzitetnem indeksu ni bilo opaznejših razlik na preučevanih lokalitetah, medtem ko je bila biomasa v povprečju dvakrat večja pod gojiščem. Grozdičasta analiza in nemetrično večdimenzionalno skaliranje (nMDS) sta pokazala očitno razliko med lokalitetama, krivulji ABC (primerjava med številčnostjo in biomaso) pa manjše motnje na gojišču školjk. Rahli učinek gojenja školjk na makrozoobentos bi lahko bil posledica zmanjšanega obsega gojenja klapavic v zadnjih letih, predvsem pa hidrodinamičnih značilnosti območja, za katere se zdi, da igrajo pomembno vlogo v disperziji biousedlin.

Ključne besede: gojenje školjk, makrozoobentos, ocena motenj, Jadransko morje

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ANALISI COMPARATIVA DI ESEMPLARI DI *MITRA ZONATA* MARRYAT, 1818 (MOLLUSCA, GASTROPODA) PROVENIENTI DA TRE AREE DEL MEDITERRANEO

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SINTESI

Nel presente lavoro sono state descritte le caratteristiche morfologiche del mollusco e della conchiglia di Mitra zonata *Marryat, 1818. Sono stati misurati e studiati 42 esemplari di tre aree di provenienza diversa: 17 da Pirano (Slovenia), 12 da Ancona (Italia) e 13 da Malaga (Spagna). E' stato evidenziato che le tre popolazioni sono ben distinte e che il rapporto L/H = 0,28 probabilmente non è sempre un parametro assoluto per determinare con certezza il sesso di questa specie.*

Parole chiave: *Mitra zonata* Marryat, 1818, Gastropoda, Mollusca, Mediterraneo

COMPARATIVE ANALYSIS OF SPECIMENS OF *MITRA ZONATA* MARRYAT, 1818 (MOLLUSCA, GASTROPODA) IN THREE MEDITERRANEAN AREAS

ABSTRACT

In the present work, the specimens and morphological characteristics of Mitra zonata *Marryat, 1818 are described. Besides, 42 specimens of three different proveniences, i.e. 17 from Piran (Slovenia), 12 from Ancona (Italy) and 13 from Malaga (Spain), have been subjected to thorough studies and measurements, which have indicated three quite distinct populations. It has been also established that the ratio L/H = 0.28 is most probably not always an absolute parameter for undisputed sex determination in this species.*

Key words: *Mitra zonata* Marryat, 1818, Gastropoda, Mollusca, Mediterranean Sea

INTRODUZIONE

La famiglia Mitridae è una delle più apprezzate dai collezionisti per la varietà di specie che la caratterizzano, anche se queste non sono così rare e pregiate come quelle delle famiglie Cypraeidae e Conidae. Essa comprende circa 150 specie, per lo più di provenienza Indopacifica, di dimensioni medie e medio-piccole, di forma stretta, allungata ed affusolata che ricorda per l'appunto la mitra: il tipico copricapo da cerimonia prelatizio, da cui il nome della famiglia. In Mediterraneo è rappresentata da poche specie e di piccole dimensioni, fatta eccezione per *Mitra zonata* Marryat, 1818, l'unica che per grandezza ed eleganza ricorda quelle tropicali.

Questo mollusco è stato fonte di interesse e di studio già dai primi del novecento, da parte di diversi autori quali Pallary (1900), Vayssière (1901) e successivamente, Vatova (1943), Radić (1969), Zavodnik (1967) e Parenzan (1970, 1971). Si tratta di una specie protetta in molti paesi mediterranei, compresi Slovenia (Lipej *et al.*, 2006) ed Italia (SIBM).

Il presente lavoro, oltre a costituire un aggiornamento il più completo possibile sulla specie, con l'aggiunta di nuovi dati originali ed una sintesi dei lavori precedenti, analizza 42 esemplari di *Mitra zonata* aventi caratteristiche morfologiche ben distinte e provenienti da tre diverse aree mediterranee: Pirano (Slovenia), Ancona (Italia) e Malaga (Spagna) (Fig. 1).

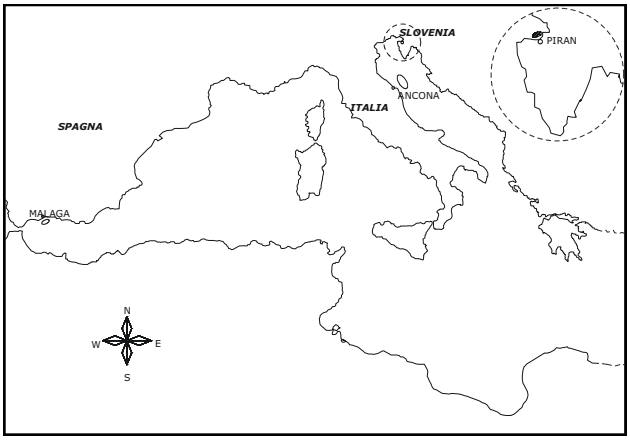


Fig. 1: Cartina geografica del Mediterraneo centro-occidentale con indicate le tree aree di raccolta.
Sl. 1: Zemljevid Sredozemlja z označenimi vzorčevalnimi predeli.

MATERIALI E METODI

Gli esemplari provenienti dall'area slovena sono stati catturati da pescatori con reti da posta trimagliate (passelere), a circa 1 miglio al largo di Pirano ed a profondità comprese tra 20 m e 30 m, mentre quelli provenienti da Ancona e da Malaga, sono stati pescati con reti a strascico, rispettivamente a 20 miglia dalla costa marchigiana, a circa 40 m di profondità ed a 10 miglia dalla costa spagnola, a circa 80 m di profondità.

Al fine di verificare le eventuali differenze morfometriche fra le tre popolazioni e le differenze di dimensioni dovute a dimorfismo sessuale all'interno della stessa popolazione, sono state rilevate le misure lineari dei singoli esemplari con un calibro digitale di tipo Mituyoto Digimatic. Tali misurazioni, espresse in millimetri, sono state eseguite secondo i parametri riportati nello schema di Figura 2.

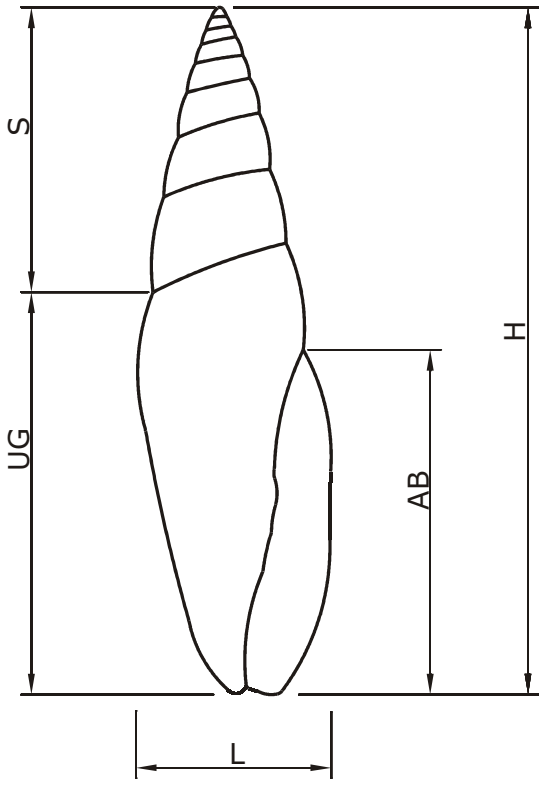


Fig. 2: Schema di** Mitra zonata **(AB – apertura boccale, H – altezza, L – larghezza, S – spira, UG – ultimo giro).
Sl. 2: Shema lupine progaste mitre (AB – ustje, H – višina, L – širina, S – svitek, UG – zadnji zavoj).

La fotografia della particolare scultura dell'apice (Fig. 3), così come quella dell'esemplare proveniente da Ancona (Fig. 4), sono state effettuate con uno Scanner mod. Epson Perfection 3200 Photo.

Per quanto concerne la sistematica ed i sinonimi è stato fatto riferimento al "*Catalogo annotato dei Molluschi Marini del Mediterraneo*" (Sabelli *et al.,* 1990). La descrizione morfologica del mollusco si riferisce agli esemplari tuttora viventi nell'acquario di Pirano, mentre quella delle conchiglie agli esemplari provenienti dalla collezione del medesimo acquario, e da quelle private dell'autrice R. De Min e del professor D. Di Massa.

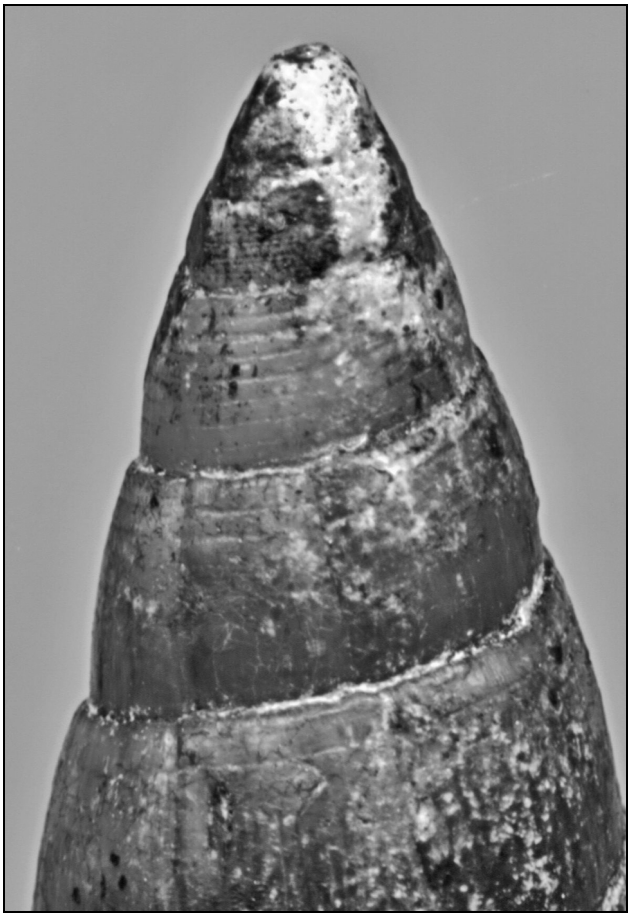


Fig. 3: Foto del particolare dell'apice.
Sl. 3: Posnetek vrha progaste mitre.

RISULTATI E DISCUSSIONE

Sistematica

Classis: GASTROPODA Cuvier, 1797
Ordo: NEOGASTROPODA Thiele, 1929
Familia: Mitridae Swainson, 1831
Genus: *Mitra* Lamarck, 1798
Species: *Mitra zonata* Marryat, 1818

Sinonimi

- *Mitra zonata* Marryat, 1818
- *Mitra zonata* Risso (Kiener, 1839)
- *Mitra santangeli* Maravigna, 1840
- *Mitra antiquata* Monterosato in Kobelt, 1874
- *Mitra zonata* var. *major* Pallary, 1900
- *Mitra zonata* var. *minor* Pallary, 1900
- *Mitra zonata* var. *protracta* Pallary, 1900
- *Mitra (Episcomitra) zonata* var. *concolor* Coen, 1934
- *Mitra zonata* var. *incurvata* Parenzan, 1970
- *Mitra fusiformis zonata* Marryat, Cernohorsky, 1970

Nella determinazione di *M. zonata* non può insorgere alcun dubbio sistematico o tassonomico, in quanto questa specie è ben distinta dalle congeneri; le differenze di taglia e di colore sono da imputarsi sia a variazioni individuali, connesse anche a dimorfismo sessuale, sia all'habitat; *M. zonata* è strettamente correlata alla specie fossile *Mitra fusiformis* (Brocchi, 1814) del Miocene (Giannuzzi Savelli, 1984).

Morfologia dell'animale

Il corpo di *M. zonata,* come quello di tutte le specie congeneri, è privo di opercolo. Il piede, spesso e relativamente lungo, presenta un muco filamentoso bianco trasparente, è tronco anteriormente ed appuntito posteriormente. Esso, bianco latte al di sotto, è fittamente puntinato di marrone-aranciato lungo il bordo, mentre la puntinatura diviene sempre più rada verso il capo e la colorazione diventa marrone più chiara, prevale sempre più un colore panna sino a raggiungere il capo che appare completamente bianco lattiginoso. La medesima sequenza di pigmentazione si può rilevare anche lungo il sifone, quest'ultimo infatti, puntinato di marrone scuro alla base, diviene sempre più chiaro fino ad essere color panna in prossimità dell'apice e quasi trasparente lungo il bordo. Il capo è caratterizzato dalla presenza di due tentacoli ben evidenti portanti ciascuno un occhio, riconoscibile come un piccolo puntino nero. L'epiproboscide, di color bruno-violaceo chiaro (Vayssière, 1901), è molto lunga, considerevolmente spessa e con l'estremità bulbosa (Sabelli, 1980). La radula è tipicamente rachiglossa, presenta cioè un dente rachidiano pluricuspidato (mediamente 7 cuspidi) e due denti laterali con circa 16–20 denticoli (Giannuzzi Savelli, 1984). Essa ha funzione predatoria e si trova per lo più in molluschi a dieta carnivora. Sebbene non si conosca ancora la specifica alimentazione di questo mollusco, è noto che altre specie di Mitridae si nutrono di sipunculidi (Moreno & Bouchet, 1998). Per quanto riguarda il ciclo vitale *M. zonata* presenta sessi separati, la fecondazione è interna e le femmine depongono le uova all'interno di una capsula ovigera che viene fissata al substrato; le larve veliger sono planctoniche. L'effettiva longevità di questa specie non è conosciuta, si sa solamente che le altre congeneri vivono fino a sei anni (Heller, 1990). Infine bisogna rilevare che, fino ad oggi, non risultano rinvenimenti di esemplari giovanili.

Descrizione della conchiglia

La conchiglia di *M. zonata* raggiunge l'altezza massima di 100 mm, ha forma ovoidale allungata e presenta suture marcate; apparentemente priva di scultura, fatta eccezione per le linee longitudinali di accrescimento ben evidenti soprattutto in prossimità dello stoma, vista al microscopio presenta una serie di minuscole fossette

allineate in modo tale da formare nei primi giri apicali 4–8 leggerissime striature spirali (Fig. 3) che, procedendo verso l'apertura boccale, vanno diminuendo fino a scomparire.

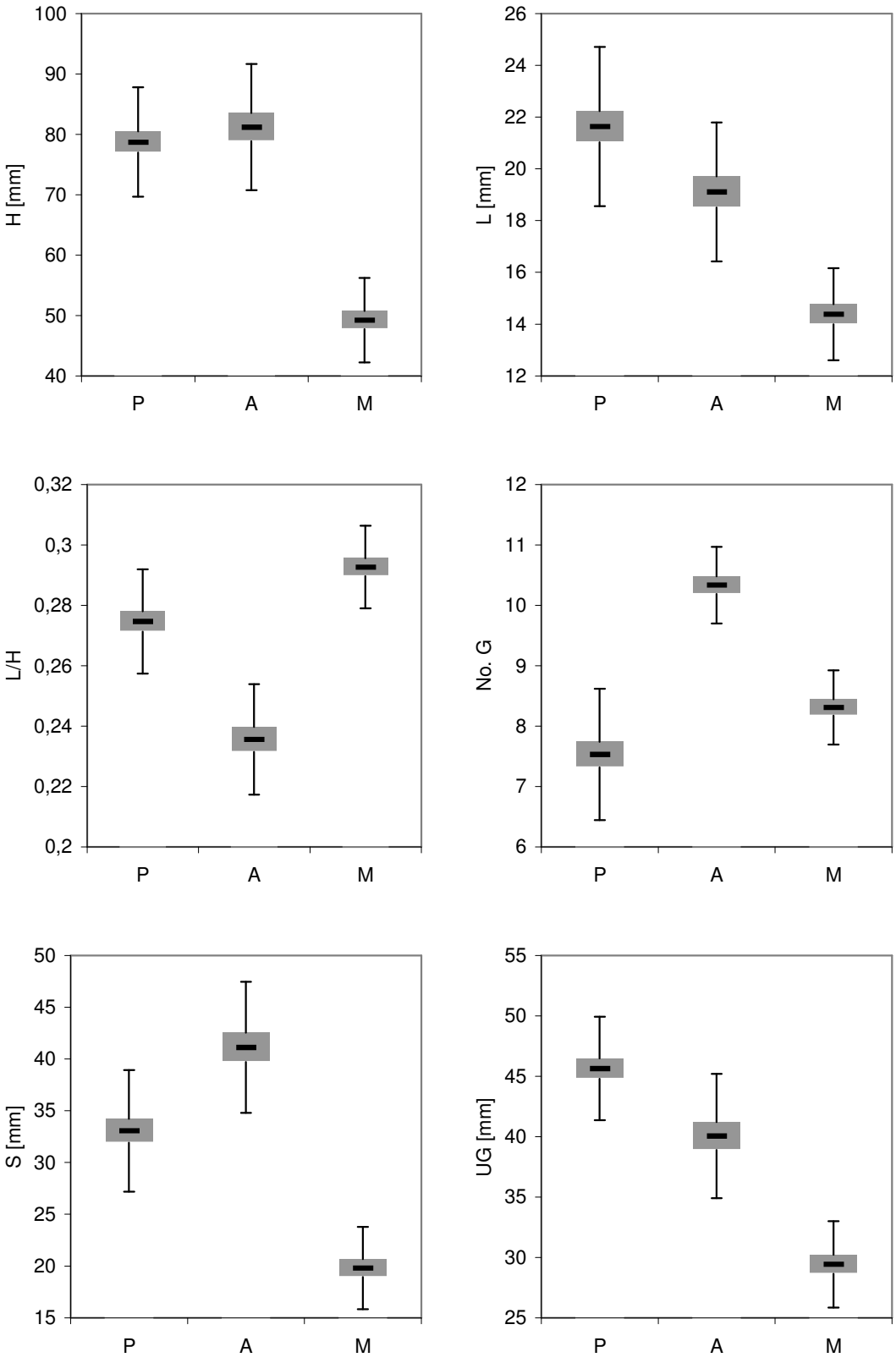
Lo stoma è caratterizzato da cinque pliche columellari crescenti in spessore dalla base verso l'apice. Tali pliche sono molto evidenti in alcuni esemplari, soprattutto in quelli pescati vivi ed integri, mentre in altri lo sono meno, tanto da non vedere la plica verso il sifone, a meno che non venga praticata una rotazione della conchiglia per esaminare l'interno dell'apertura boccale. Quest'ultima dalla parte del labbro columellare è bianca alla base con macchie marroni verso la sutura, mentre dalla parte del labbro esterno, liscio ed assottigliato, è completamente bianco-lucida. La colorazione esterna è solitamente bruno chiara, caratterizzata da una minutissima ragnatela di linee crema più o meno evidenti e da una fascia marrone scuro-nera che, nell'ultimo giro, è tanto ampia da occupare quasi tutta l'altezza dell'apertura boccale, mentre lungo la spira si riduce ad una linea che segue la sutura. Questa colorazione così caratteristica è in realtà quella del periostraco corneo, infatti, una volta decorticato, la conchiglia risulta color crema con una banda sfumata color ruggine (Ferrario, 1991).

Osservazioni e studi biometrici

Attualmente nell'acquario di Pirano vivono due esemplari di *M. zonata* uno da circa 20 mesi, l'altro da due anni e mezzo. In passato l'acquario aveva ospitato altri esemplari che erano sopravvissuti per circa due mesi. Tale prolungamento della vita in cattività è sicuramente riconducibile all'aggiunta di sedimento nella vasca che ospita gli esemplari. *M. zonata*, infatti, vive semi-infossata lasciando sporgere il sifone e la parte superiore della spira; questo motivo, e la minore profondità in cui vive, può in parte giustificare la corrosione riscontrata sulla zona dorsale della spira di tutti gli esemplari pescati a Pirano, i quali non sono mai stati rinvenuti con il periostraco integro. Le conchiglie provenienti da Pirano e da Malaga rispecchiano il colore tipico della specie, anche se quelle di Malaga hanno il periostraco integro e più lucido rispetto a quelle di Pirano. Gli esemplari provenienti da Ancona hanno un colore molto più chiaro: giallo-verde oliva non marmoreggiato con la fascia marrone chiaro-nocciola; la forma della conchiglia è particolare soprattutto perché presenta l'apice lievemente incurvato (Fig. 4).

Parenzan, pensando ad una razza comparsa per mutazione, ritenne opportuno indicarla come varietà: *M. zonata* var. *incurvata* Parenzan, 1970 (Parenzan, 1970). L'ipotesi del Parenzan, che la fossa al largo di Ancona ospiti una varietà a parte, è suffragata dai dati riportati in Figura 5. Osservando la tabella, infatti, si nota che negli esemplari provenienti da Pirano e da Malaga la misura della spira è sempre minore rispetto a quella dell'ultimo giro (S < UG) e di conseguenza il rapporto S/UG è sempre minore di 1 (S/UG < 1), così come, eccetto in un unico caso per Pirano (AB = 33,56 mm), le dimensioni dell'apertura boccale sono sempre maggiori di quelle della spira (AB > S). Negli esemplari di Ancona la spira è molto spesso maggiore o di poco minore all'ultimo giro con un conseguente rapporto S/UG quasi sempre maggiore di 1 o di poco inferiore e l'apertura boccale è sempre minore della spira (AB < S). Da ciò si deduce che gli esemplari da Ancona differiscono dagli altri anche nella maggiore altezza e che questa è data soprattutto da una spira più alta; ciò è confermato anche dal numero di giri che per Pirano e per Malaga sono al massimo 9, in accordo con gli studi effettuati da altri autori, mentre per Ancona sono sempre 10 o 11: gli esemplari di Ancona presentano, quindi, una conchiglia variabile non solo nel colore e nella forma ma anche nelle dimensioni.

Fig. 4: Foto di un esemplare di** M. zonata **proveniente da Ancona (apice incurvato).
Sl. 4: Posnetek primerka progaste mitre z ukrivljenim vrhom iz okolice Ancone.


H [mm]
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26
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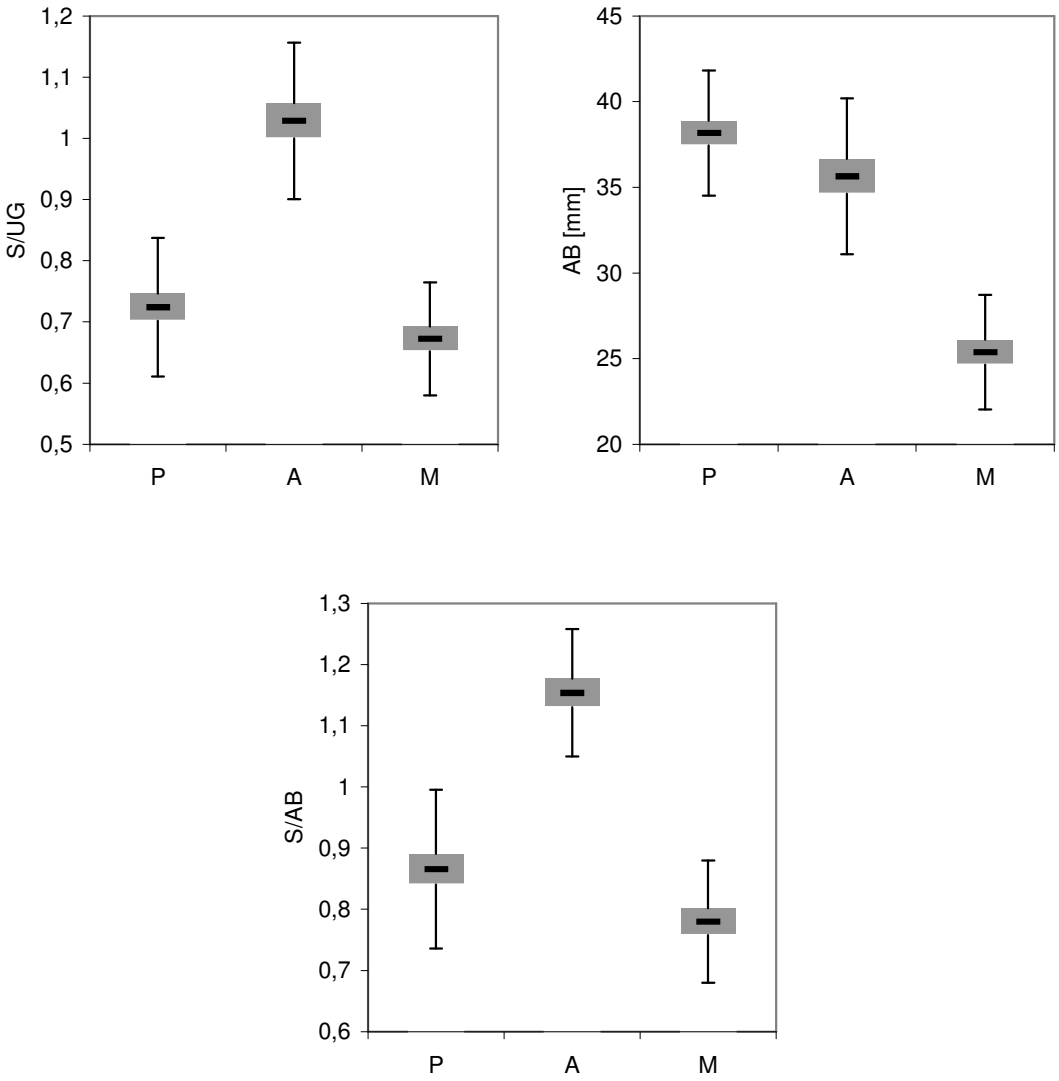

Fig. 5: Parametri biometrici delle conchiglie di** M. zonata **in tre aree mediterranee (P – Pirano, A – Ancona, M – Malaga, AB – apertura boccale, H – altezza, L – larghezza, S – spira, UG – ultimo giro, No G – numero dei giri).
Sl. 5: Biometrični parametri, izmerjeni na lupinah progaste mitre v treh sredozemskih predelih (P – Piran, A – Ancona, M – Malaga, AB – ustje, H – višina, L – širina, S – svitek, UG – zadnji zavoj, No G – število zavojev).

Analizzando ulteriormente i dati riportati in Figura 5 e Figura 6, risulta evidente che le tre popolazioni sono ben distinguibili: le conchiglie di Ancona sono più alte, soprattutto in rapporto a quelle di Malaga, tanto che l'esemplare più basso di Ancona (H = 64,20 mm) risulta comunque più alto dell'esemplare di maggiori dimensioni di Malaga (H = 60,65 mm); si può osservare, inoltre, che il rapporto L/H negli esemplari di Ancona è compreso in un intervallo di valori tra $0{,}213 \leq L/H \leq 0{,}256$, ed il valore massimo (L/H = 0,256) risulta essere decisamente al di sotto del valore minimo di Malaga (L/H = 0,281) che risulta compreso tra i valori $0{,}281 \leq L/H \leq 0{,}308$. Tra queste due popolazioni limite, Pirano presenta esemplari con maggior variabilità di altezza e del rapporto L/H che risulta compreso tra i valori $0{,}246 \leq L/H \leq 0{,}296$. La spiccata differenza di dimensioni tra le tre popolazioni e di variabilità del rapporto L/H, all'interno della medesima popolazione, è evidenziato in Figura 6.

Pallary (1900) e Vayssière (1901) notarono già una variabilità del rapporto L/H in *M. zonata*. Vaiyssière (1901), inoltre, constatò sperimentalmente, dall'esame anatomico di alcuni esemplari, che i maschi presentavano dimensioni maggiori, con forme molto elate e strette, mentre le femmine erano relativamente più corte e più panciute. Questa specie mostrava, pertanto, uno

spiccato dimorfismo sessuale che si manifestava principalmente attraverso la notevole variabilità del rapporto L/H. Tale rapporto, dall'esame dei dati di 40 esemplari, risultava compreso tra 0,22 e 0,34, con una media pari a 0,28. Sostanzialmente se il rapporto risulta L/H ≤ 0,28 gli esemplari sono maschi (indicati in Fig. 6 con M), se risulta L/H ≥ 0,28 sono femmine (indicati in Fig. 6 con F). Alla luce di quanto esposto è facile ipotizzare come la varietà *minor* Pallary, 1900 (H = 29 mm, L = 10 mm, L/H = 0,34) possa in realtà riferirsi ad esemplari di sesso femminile, mentre la varietà *Mitra antiquata* Monterosato in Kobelt, 1874 (H = 90 mm, L = 20 mm, L/H = 0,22), possa invece riferirsi a quelli di sesso maschile (Giannuzzi Savelli, 1984); benché nella maggior parte dei casi il rapporto L/H ≤ 0,28 corrisponda sicuramente ad esemplari di sesso maschile e L/H ≥ 0,28 ad esemplari di sesso femminile, in alcuni casi il limite del rapporto andrebbe forse rivisto in funzione dell'habitat particolare in cui vive il mollusco.

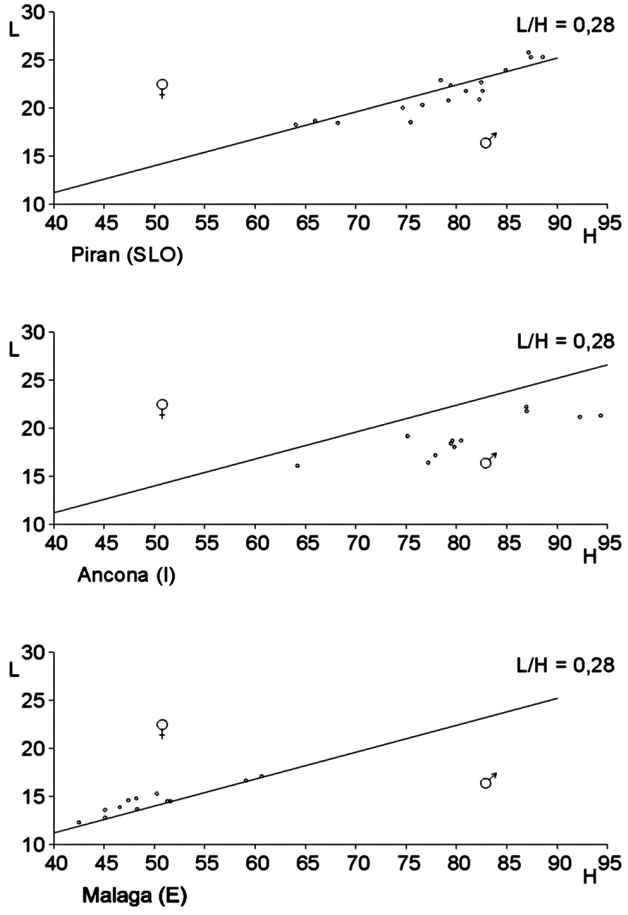

Fig. 6: Rapporto tra la larghezza (L) e l'altezza (H) delle conchiglie di** M. zonata **provenienti da tre aree mediterranee.
Sl. 6: Odnos med širino (L) in višino (H) pri lupinah progaste mitre iz treh sredozemskih predelov.

Analizzando, infatti, i dati ottenuti dalle misurazioni di 17 esemplari provenienti da Pirano, 12 da Ancona e 13 da Malaga è stato rilevato che, mentre per gli esemplari di Pirano le teorie di Giannuzzi Savelli (1984) sono confermate, infatti il 53% sono maschi ed il 47% femmine (Fig. 5), ciò non sembra valido per quelli di Ancona e di Malaga, in cui il rapporto L/H risulta sempre inferiore a 0,28 per i primi e gli esemplari sono o dovrebbero essere tutti maschi, mentre per i secondi il rapporto L/H risulta sempre superiore a 0,28 ed in questo caso gli esemplari sono o dovrebbero essere tutti femmine. Bisogna considerare il fatto che il numero di conchiglie misurate è relativamente basso, ma vista la rarità della specie, risulta comunque superiore a quello degli studi precedenti. Appare improbabile che fra gli esemplari di Ancona non vi sia nemmeno una femmina e viceversa per Malaga. Sommando tutti i valori L/H degli esemplari di Pirano e calcolando la media ($\overline{m}$), questa risulta $\overline{m}$ = 0,275 che, nonostante i pochi dati, è un valore prossimo a 0,28. Sommando invece i valori L/H degli esemplari di Ancona e di Malaga si nota che la media è rispettivamente $\overline{m}$ = 0,235 ed $\overline{m}$ = 0,292, ovvero valori relativamente discosti da 0,28, soprattutto per Ancona. Si può ipotizzare, pertanto, che il valore L/H = 0,28 dovrebbe essere diminuito per gli esemplari provenienti da Ancona e leggermente aumentato per quelli provenienti da Malaga, visto che si tratta di due popolazioni limite in termini di grandezze lineari. Al fine di validare tali ipotesi sono necessari ulteriori studi di approfondimento. Ad esempio, se si pone L/H = 0,23 per Ancona e si analizzano i dati riportati in Figura 5, secondo questo nuovo parametro (M ≤ 0,23 ≤ F) risultano sette esemplari con L/H ≥ 0,23, e quindi femmine, e cinque esemplari con L/H ≤ 0,23, ovvero maschi. Ponendo invece L/H = 0,29 per Malaga (M ≤ 0,29 ≤ F), risultano cinque femmine e sette maschi; in entrambi i casi i dati sono più o meno comparabili a quelli di Pirano. Come già evidenziato, i parametri L/H = 0,23 per Ancona e L/H = 0,29 per Malaga sono stati solamente ipotizzati in base alle medie calcolate dai dati in possesso ed ulteriori studi, soprattutto anatomici, potrebbero confermarli o modificarli. Da quanto esposto risulta in ogni caso evidente che il parametro L/H = 0,28 non è sempre corretto per rilevare con certezza il sesso del mollusco dalle sole misurazioni delle conchiglie. È noto infatti che, molto spesso, la conchiglia dei gasteropodi si presenta leggermente o significativamente modificata nella forma e nelle dimensioni a seconda dall' habitat in cui vive. Questo non esclude il fatto che all'interno della medesima popolazione, seppur con parametri diversi, gli effetti del dimorfismo sessuale si riflettono comunque sul fenotipo.

Distribuzione e biocenosi

Per quanto riguarda la distribuzione geografica di *M. zonata* è doveroso ricordare Vatova (1943), l'autore che per primo ne studiò le provenienze in Mediterraneo. Questa specie si rinviene nel Mediterraneo centro-occidentale, ma sembra essere assente nel bacino orientale (Giannuzzi Savelli, 1984). E' più frequente nel Mare Adriatico (D'Angelo & Gargiullo, 1978), soprattutto nel bacino centrale e settentrionale, e nel Mare di Alboran al largo di Malaga. Per quanto riguarda l'Atlantico la si rinviene dalle Isole Azzorre al Portogallo (Burnay & Martins, 1988), dalle Canarie (Nordsieck & Garcìa-Talavera, 1979) e Madeira (Abreu, 1991) al Marocco ed alla Mauritania. Vive generalmente a profondità che si aggirano tra i 50-150 m anche se nell'Alto Adriatico si trova a profondità minori: 20-30 m, e nell'Oceano Atlantico è stata rinvenuta fino a 1250 m (Poppe & Goto, 1991). È presente nella biocenosi dei fondi detritici costieri (DC) (Ghirardelli, 1981), ma più di frequente in quella dei fondi detritici fangosi (DF) (Vio & De Min, 1996) del piano circalitorale. Tale specie, infatti, è stata raccolta a 50–70 m di profondità su un fondale ricco di *Peyssonnelia polymorpha* (Parenzan, 1971), alga calcarea tipica della facies ad ascidie che prospera su fondi formati da fanghi molto fluidi (Ghirardelli, 1981). Zavodnik (1967) ha ritrovato *M. zonata* a 30 m di profondità su fondo fangoso caratterizzato dalla presenza di *Ophiothrix*, inoltre Coen (1934) ha rinvenuto diversi esemplari, di colore uniforme bruno scuro (*Mitra concolor* Coen, 1934 var.), ad una profondità di 30 m su fondo fangoso presso l'isola Figarola nell'Alto Adriatico (Parenzan, 1971). Infine Radić (1969) menziona esemplari provenienti dalla Croazia (Rovinj, Hvar, ed altri siti), raccolti a profondità diverse, principalmente su fondale fangoso-sabbioso.

CONCLUSIONI

Mitra zonata deve la fama non solo alla bellezza della conchiglia, ma anche alla sua rarità, benché negli ultimi anni sembra essere più frequente soprattutto nell'Alto Adriatico, dove evidentemente trova condizioni ambientali particolarmente favorevoli. Il numero più elevato di ritrovamenti è dovuto anche ad una maggiore informazione da parte dei pescatori che attualmente, sapendo che questa specie è ricercata dai collezionisti, pongono molta più attenzione durante la pulizia delle reti. Inoltre, rispetto ad un tempo, sembra essersi avvicinata alla costa: ora infatti si rinviene anche nel Golfo di Napoli, mentre tra il 1931 ed il 1936 Parenzan (1971), pur effettuando un migliaio di dragaggi, non raccolse nemmeno un esemplare. Nonostante ciò, questa specie è stata inserita da tempo nell'elenco delle specie marine protette in Italia edito dalla Società Italiana di Biologia Marina (S.I.B.M.) ed in Slovenia (Lipej *et al.*, 2006).

Nel presente lavoro sono state condotte analisi biometriche su 42 esemplari di *M. zonata* provenienti da Pirano (SLO), Ancona (I), Malaga (E). Dallo studio è emerso che le tre popolazioni sono distinte e presentano caratteristiche diverse. *M. zonata*, come molte altre specie di gasteropodi, presenta un adattamento all'habitat che si esprime non solo nel colore e nella grandezza, ma anche nella morfologia della conchiglia e ciò risulta ben evidente soprattutto negli esemplari provenienti da Ancona. È una specie a sessi separati il cui dimorfismo sessuale, che si riflette sul fenotipo nelle dimensioni della conchiglia, è strettamente correlato al rapporto L/H. Questo rapporto, però, non può essere considerato un parametro fisso (L/H = 0,28), al di sotto ed al di sopra del quale gli esemplari possono essere riconosciuti con certezza come maschi o femmine. Il suo adattamento all'ambiente, a fronte della carenza, dell'abbondanza, della qualità del cibo e/o delle variazioni dei parametri chimico-fisici dell'acqua, si riflette sullo sviluppo dimensionale delle conchiglie nelle singole popolazioni.

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PRIMERJALNA ANALIZA OSEBKOV PROGASTE MITRE, *MITRA ZONATA* MARRYAT, 1818 (MOLLUSCA, GASTROPODA), V TREH PREDELIH SREDOZEMSKEGA MORJA

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POVZETEK

Avtorji opisujejo morfološke značilnosti osebkov in lupin progaste mitre, Mitra zonata *Marryat, 1818, nabranih v treh predelih Sredozemskega morja. Progasta mitra je razmeroma velik polž iz družine Mitridae, sicer pa povsod redka. Zato je danes na seznamu ogroženih vrst v številnih sredozemskih državah. Avtorji so analizirali 42 osebkov progaste mitre: 17 iz okolice Pirana (Slovenija), 12 iz okolice Ancone (Italija) in 13 iz okolice Malage (Španija). Na podlagi biometričnih meritev se je izkazalo, da se polži med seboj dobro ločijo. Obenem se je izkazalo, da korelacijski odnos med širino in višino (L/H = 0,28) ni dober razmejitveni faktor za ugotavljanje spola te vrste.*

Ključne besede: *Mitra zonata* Marryat, 1818, Gastropoda, Mollusca, Sredozemsko morje

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EVIDENCE OF PREDATORY ATTACK ON A BOTTLENOSE DOLPHIN *TURSIOPS TRUNCATUS* BY A GREAT WHITE SHARK *CARCHARODON CARCHARIAS* IN THE MEDITERRANEAN SEA

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ABSTRACT

A live adult bottlenose dolphin Tursiops truncatus, *bearing two fresh bites of great white shark* Carcharodon carcharias *on its dorsal region, was encountered on May 5, 2006 near Lampedusa, Italy, Mediterranean Sea. It is estimated that the wounds suffered by this mammal were inflicted by a white shark greater than 4 meters in length. This is the first time that a bottlenose dolphin that survived a white shark attack has been reported from the Mediterranean, and is definitive evidence that* C. carcharias *actually attacks live bottlenose dolphins in these waters.*

Key words: bottlenose dolphin, *Tursiops truncatus*, great white shark, *Carcharodon carcharias*, predation

TESTIMONIANZA DI ATTACCO PREDATORIO SU TURSIOPE *TURSIOPS TRUNCATUS* DA PARTE DI SQUALO BIANCO *CARCHARODON CARCHARIAS* NEL MARE MEDITERRANEO

SINTESI

Un tursiope Tursiops truncatus *adulto, recante recenti ferite causate da due morsi di squalo bianco* Carcharodon carcharias *nella regione dorsale, è stato incontrato il 5 Maggio 2006 al largo di Lampedusa, Italia, Mare Mediterraneo. Si stima che le ferite siano state inferte dal cetaceo da parte di uno squalo bianco di oltre 4 metri di lunghezza. Questa è la prima volta che un tursiope sopravvissuto ad un attacco di squalo bianco viene segnalato nel Mediterraneo, ed è la prova definitiva che* C. carcharias *attacca effettivamente tursiopi vivi in queste acque.*

Parole chiave: tursiope, *Tursiops truncatus*, squalo bianco, *Carcharodon carcharias*, predazione

INTRODUCTION

Cetacean remains are frequently found in the stomachs of great white sharks *Carcharodon carcharias* (Linnaeus, 1758), but a fundamental distinction must be made between scavenging a dead cetacean and the act of predation, by attacking or killing a live cetacean. Of over 479 species of sharks (Compagno, 2001), only about 6, including the great white shark, are known to prey on small odontocetes (Long & Jones, 1996). White shark's bite scars and fresh wounds have been used with success to identify the species of sharks responsible for predation and scavenging on humans, pinnipeds, cetaceans, and other animals (Cigala Fulgosi, 1990; Long & Jones, 1996; Long *et al.*, 1996; Collier, 2003). In the Mediterranean Sea, white sharks feed on cetaceans, including bottlenose dolphins *Tursiops truncatus* (Montagu, 1821), common dolphins *Delphinus delphis* Linnaeus, 1758, sperm whales *Physeter macrocephalus* Linnaeus, 1758, and fin whales *Balaenoptera physalus* (Linnaeus, 1758) (De Maddalena, 1999, 2002, unpubl. data), but white shark predation on cetaceans is poorly documented (Fergusson, 1994, 1996; Bianucci *et al.*, 2002). Presented here is the first case of a white shark-bitten, live bottlenose dolphin *Tursiops truncatus* from the Mediterranean.

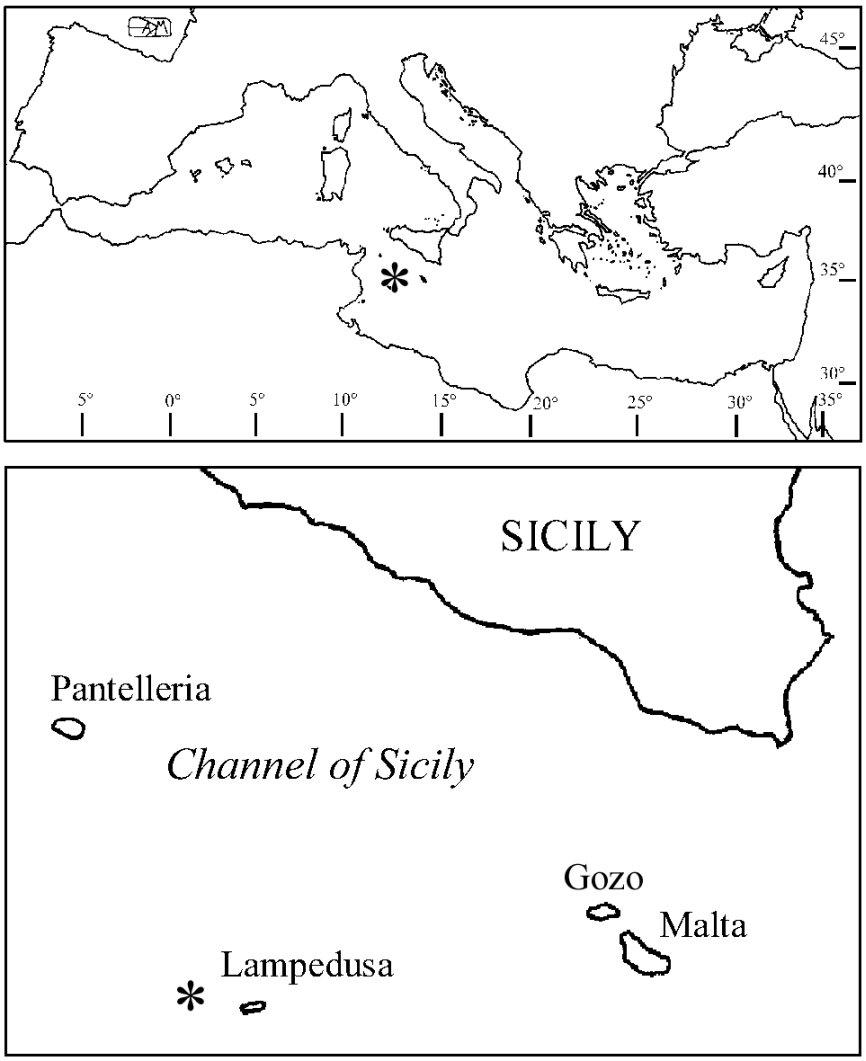

Fig. 1: Area of the Central Mediterranean Sea showing the location where a bottlenose dolphin *Tursiops truncatus* ***bearing two fresh bites of great white shark*** *Carcharodon carcharias* ***was encountered. (Drawing: A. De Maddalena) Sl. 1: Območje osrednjega dela Sredozemskega morja z lokacijo, na kateri je bila opažena velika pliskavka*** *Tursiops truncatus* ***z dvema svežima ugrizoma, ki ji ju je prizadejal beli morski volk*** *Carcharodon carcharias.* ***(Risba: A. De Maddalena)***

MATERIAL AND METHODS

Since 1996, the Italian Great White Shark Data Bank (Banca Dati Italiana Squalo Bianco) has collected a substantial amount of information regarding historical and recent records of the great white shark from the Mediterranean Sea. This data includes information on size, distribution, habitat, behaviour, reproduction, diet, fishery and attacks on humans (De Maddalena, 1998, 1999, 2000a, 2000b, 2002, 2005; Celona *et al.*, 2001; De Maddalena *et al.*, 2001, 2003; Galaz & De Maddalena, 2004). The study of the trophic relationships between great white sharks and the Mediterranean fauna is a fundamental part of this research program.

The observation reported in this work was made during a research program in the area of the Isole Pelagie, Italy, where Necton Marine Research Society manages and maintains a study of the marine mammals that regularly frequent the archipelago waters. The observations reported here were made near Lampedusa (35° 28,912 N, 12°33,865 E) (Fig. 1). Surface and underwater observations were made during favourable weather conditions (sea state Beaufort 1). Surface photographs were taken using a Canon EOS 350D.

RESULTS AND DISCUSSION

On May 5, 2006, in the late afternoon, at approximately 17.00 hrs, two of the authors (A.C. and G.C.) were aboard a 6 m inflatable boat off Lampedusa. Nearby, four bottlenose dolphins were observed surfacing. The cetaceans travelled relatively slowly with their dorsal fins protruding above the surface. The group consisted of three adults and one calf that moved in two subgroups, comprised of two adults, and one adult with one calf, respectively. The dolphins followed a fishing boat that was fishing with a bottom trawl. The dolphins swam about 500 m from the boat stern, performing dives of four minutes. The subgroup formed by the two adults was about 50 m in front of the second subgroup. The dolphins never allowed the observers to approach them closer than 100 m. As the observers moved towards the dolphins, the cetaceans moved farther away or momentarily swam deeper out of visibility range and then reappeared on the opposite side of the boat. One of the adult bottlenose dolphins, approximately 3.5 m in length, showed fresh wounds from two shark bites on its dorsal region (Fig. 2). The whole encounter lasted 30 minutes in total.

By closely examining the wounds, the authors were able to identify the species of shark responsible for the attack as a great white shark. The wounds were located in the dolphin's dorsal region, and had a wide parabolic shape, suggesting an extremely wide mouth. The dorsal fin showed an evident bite mark on its anterior margin. The wounds were mainly made of regular marks, inflicted by the white shark's large, triangular, serrated, well-spaced teeth. We believe the slice, or cut, in front of the dorsal fin is a bite from a white shark's lateral teeth. The other wound, which consists of the five distinct punctures below the dorsal fin, especially their spacing, is very suggestive of upper anterior teeth of a white shark. Counting two punctures down from the uppermost wound, the distance between the second and third punctures appears to be the centre of the jaw with the small cut, number five from the top, being the

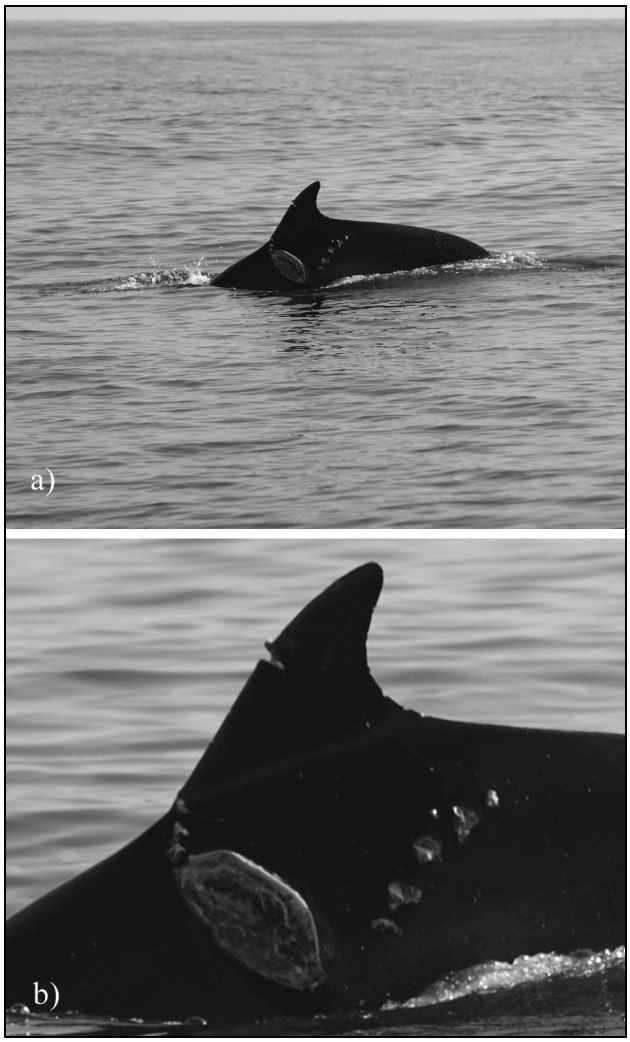

Fig. 2: (a) Estimated 3.5 m bottlenose dolphin** T. truncatus**, photographed on May 5, 2006, near Lampedusa, Isole Pelagie, Italy. (b) Close-up of the dolphin's dorsal region that shows two fresh white shark bites. (Photo: E. D'Andrea)

Sl. 2: (a) Približno 3,5 m dolga velika pliskavka** T. truncatus**, fotografirana 5. maja 2006 nedaleč od otoka Lampedusa v Pelagijskem otočju (Italija). (b) Na bližnjem posnetku hrbtne strani velike pliskavke sta lepo vidna sveža ugriza belega morskega volka. (Foto: E. D'Andrea)

intermediate, or third tooth over from the centre of the jaw. This tooth is smaller than the first and second anterior, with the tip of the tooth pointing inwards toward the second anterior. The 'interspace' between the individual wounds also suggests the animal was greater than 4 meters in length.

The great white shark often suddenly attacks the fast-swimming animals on which it feeds; the success of this predatory strategy depends on the element of surprise (Strong, 1996). It is thought that the great white shark would have difficulty capturing a healthy, fast-swimming prey item if the animal were aware of the predator's presence. The victim typically never sees the shark until it is too late and is overwhelmed by the unexpected assault and the violent force with which it is executed. These sharks have been reported to attack seals, sea lions, sea otters, dolphin, tuna, and human beings using this method (Miller & Collier, 1980; Tricas & McCosker, 1984, Ames *et al.*, 1996; Levine, 1996; Long & Jones, 1996; Long *et al.*, 1996; West, 1996; Collier, 2003). We conclude that the great white shark bit the bottlenose dolphin during a surprise attack, inflicting two bites on it.

White shark approaches on a prey item can be oriented horizontally or vertically. The predator uses its heavy mass and speed to violently ram, disorient, and stun the prey. During vertical approaches, the white shark attacks its prey from below, swimming from depths as deep as 17 metres and moving on a line that is at an angle of 45-90° from the prey (Strong, 1996). The bottlenose dolphin has anteriorly directed sonar and a lateral visual field, so a surprise attack must be in the blind area, either from above, below, or behind to avoid detection (Long & Jones, 1996). We believe that the white shark approached from above and the side the bottlenose dolphin.

White sharks focus their bites on particular areas of the odontocete body: the caudal peduncle, the urogenital region, the abdominal area, and the dorsum. A bite in the first three cited regions may immediately immobilize or kill the cetacean. The dorsal region has a thicker blubber and muscle mass, and a bite in this area would less likely cause death, therefore healed shark wounds are more frequently seen on the backs of living odontocetes (Long & Jones, 1996). The wounds we observed on the dorsum of the bottlenose dolphin were certainly serious and very fresh.

Other 24 cases of trophic interactions between white sharks and dolphins in the Mediterranean have been collected by the Italian Great White Shark Data Bank, but this is the first time that a bottlenose dolphin that survived a white shark attack has been reported from the Mediterranean. Therefore the case reported is the definitive evidence that *C. carcharias* actually attacks live bottlenose dolphins in the Mediterranean.

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PLENILSKI NAPAD BELEGA MORSKEGA VOLKA *CARCHARODON CARCHARIAS* NA VELIKO PLISKAVKO *TURSIOPS TRUNCATUS* V SREDOZEMSKEM MORJU

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POVZETEK

Dne 5. maja 2006 je bila v bližini Lampeduse (Italija, Sredozemsko morje) opažena živa odrasla velika pliskavka Tursiops truncatus *z dvema svežima ugrizoma na hrbtu. Avtorji članka ocenjujejo, da je rane temu vodnemu sesalcu*

prizadejal več kot 4 metre dolg beli morski volk Carcharodon carcharias. *To je prvo poročilo iz Sredozemskega morja o veliki pliskavki, ki je preživela napad belega morskega volka, in nedvomen dokaz, da* C. carcharias *resnično napada žive velike pliskavke v teh vodah.*

Ključne besede: velika pliskavka, *Tursiops truncatus*, beli morski volk, *Carcharodon carcharias*, plenilstvo

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SKATES AND RAYS (CHONDRICHTHYES) FROM WATERS OFF THE LANGUEDOCIAN COAST (SOUTHERN FRANCE, NORTHERN MEDITERRANEAN): A HISTORICAL SURVEY AND PRESENT STATUS

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ABSTRACT

A literature review based on ichthyological papers published between 1860 and 1965 shows that 22 skate and rays species occurred off the Languedocian coast (southern France, northern Mediterranean). Investigations conducted since 1988 to date allow to state that only 12 species occurred in the area, 2 of which could be considered abundant and 3 relatively common. The other species were occasionally landed at fishing sites. The decline of captures may be due to fishing pressure.

Key words: Chondrichthyes, skates, rays, Languedocian coast, Mediterranean

RAZZE (CHONDRICHTHYES) AL LARGO DELLA COSTA DI LANGUEDOC (FRANCIA MERIDIONALE, MEDITERRANEO SETTENTRIONALE): REVISIONE STORICA E STATO ODIERNO

SINTESI

L'articolo presenta una revisione storica basata su articoli inerenti l'ittiologia, pubblicati fra il 1860 ed il 1965. Secondo tali articoli, 22 specie di razze sono state avvistate al largo della costa di Languedoc (Francia meridionale, Mediterraneo settentrionale). Studi condotti dal 1988 ad oggi confermano il ritrovamento di sole 12 specie nell'area in questione, delle quali 2 possono venir considerate abbondanti e 3 relativamente comuni. Le restanti specie sono occasionalmente arrivate nelle zone di pesca. Il declino nelle catture potrebbe essere dovuto a un'eccessiva pressione di pesca.

Parole chiave: Chondrichthyes, razze, costa di Languedoc, Mediterraneo

INTRODUCTION

A literature review showed that information on skates and rays from the Languedocian coast did not constitute the focus of a special report. Their occurrence in the area was only included in faunistic publications from Doumet (1860) to Quignard *et al.* (1962). It was only Quignard (1965) who provided observations on rajid species and Capapé *et al.* (2006c) who described the discovery of the speckled ray, *Raja polystigma* Regan, 1923.

However, recent investigations conducted since 1988 allow reporting observations carried out on sharks from waters off the Languedocian coast (Capapé *et al.*, 2000b). In this paper we present similar data on skates and rays concomitantly collected in the same area. Moreover, we propose herein a historical survey of species reported formerly and species collected recently in order to carry out a comparison between previous and recent occurrence.

Both general and local distribution, abundance, bio-ecological data and some available traits of their reproductive biology are given in the article.

MATERIAL AND METHODS

Data provided in this paper were collected in a literature review with special regard to the Languedocian coast. Investigations were conducted in the area especially at fish harbours of Sète, Le Grau du Roi and Aigues-Mortes, and at the fishing sites of Palavas-Les-Flots and Carnon (Fig. 1). The observed specimens were captured by trawling and/or commercial gill-nets. Moreover, research surveys were conducted in the same areas on board the oceanographic trawler 'Georges Petit', in November 1988 and 1990 and May 1992 and 1993.

For both, scientific and vernacular names (in English and French) were given, following Whitehead *et al.* (1984). Total length and/or disc width were measured to the nearest millimetre, following Clark (1926) and Capapé *et al.* (2004), mass was assessed to the nearest gram, when possible.

RESULTS

Family Pristidae

Pristis pristis (Linnaeus, 1794): common sawfish (En), poisson-scie commun (Fr)

According to Tortonese (1956), *Pristis pristis* was reported in the eastern Atlantic from Portugal to Angola. In the Mediterranean, it was also reported off Spain and Balearic Isles (Lozano Rey, 1928), off Sicily (Tortonese, 1956), but Tortonese (1987) considered its occurrence doubtful in the area.

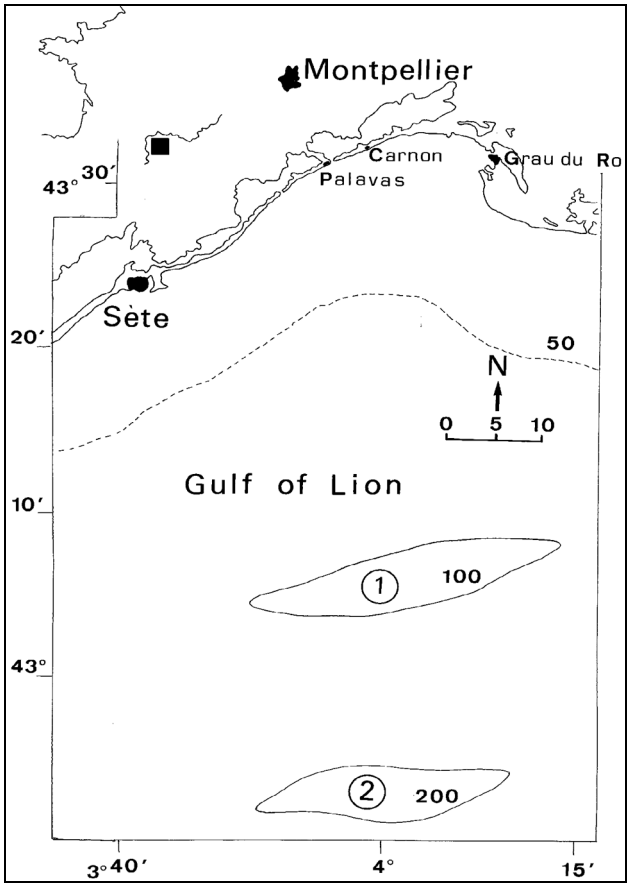

Fig. 1: Map of France with the coast of Languedoc and the 'pits' off Sète, where the small spotted catshark, *Scyliorhinus canicula* ***(1) and the blackmouth catshark*** *Galeus melastomus* ***(2) are the dominant elasmobranch species. (Redrawn from Capapé*** *et al.****, 2000b)***
Sl. 1: Zemljevid Francije z obalnimi vodami Languedoca in "votlinami" v bližini Sèteja, kjer sta navadna morska mačka *Scyliorhinus canicula* ***(1) in morska mačka*** *Galeus melastomus* ***(2) prevladujoči vrsti iz podrazreda morskih psov in skatov. (Narisano po Capapé*** *et al.****, 2000b)***

Off the Languedocian coast, the species was formerly cited by Moreau (1881) who has not recorded specimens and referred to previous observations. Granier (1964) noted that the species could reach about 4 metres in total length and was occasionally captured in the area. He added that the last common recorded sawfish (1,500 mm in total length) was captured in October 1959. No further capture has been made in the area to our knowledge, to date.

Family Rhinobatidae

Rhinobatos rhinobatos (Linnaeus, 1758): common guitarfish (En), guitare de mer commune (Fr)

Granier (1964) noted that the common guitarfish was occasionally captured by trawlers off the Languedocian coast. The captured specimens had 1m total length maximum. No recent capture of the species has been recorded to date.

Family Torpedinidae

Torpedo marmorata Risso, 1810 (Fig. 2): marbled electric ray (En), torpille marbrée (Fr)

The marbled electric ray was reported in the eastern Atlantic from British Isles (Wheeler, 1969) to the Gulf of Guinea (Blache *et al.*, 1970). The species was recorded in tropical areas but landings are rather rare, especially off the coast of Senegal (Séret & Opic, 1990; Capapé *et al.*, 2001). In the area, males and females were adult over 270 mm and 380 mm in total length respectively (Capapé *et al.*, 2001). Southwards, the species is recorded off the coasts of Southern Africa (Smith & Heemstra, 1986). *T. marmorata* is reported throughout the Mediterranean, however, captures are more abundant in the western basin and off the northern shore. In Tunisian waters, the species is considered rare by Capapé (1979), who noted that the species was rather captured off Cape Bon, in northern areas, moreover, recent data allowed to show that the species was also captured in the Gulf of Tunis and also entered Tunis Southern Lagoon (Mejri *et al.*, 2004).

Off the coast of Languedoc, *T. marmorata* was reported by Doumet (1860), Moreau (1881), Calvet (1905), Euzet (1959) and Quignard *et al.* (1962). It was captured by gill-netters concomitantly with *T. torpedo*, but landings occurred abundantly every day. Observations bases on several hundred specimens, males and females, showed that in 221 males total length ranged from 160 to 400 mm and weighed from 40 to 1,000 g, while total length of females ranged from 210 to 500 mm and mass from 230 to 3,500 g.

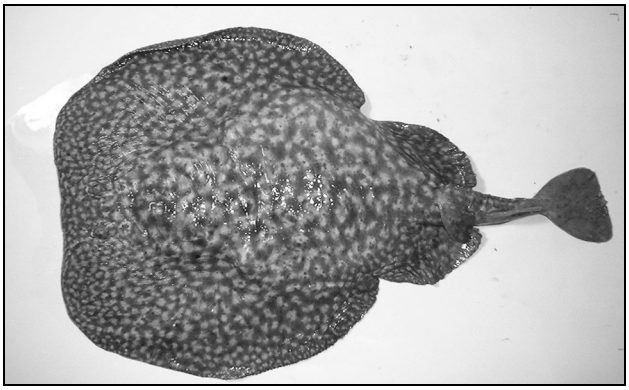

Fig. 2: *T. marmorata* ***captured off the Languedocian coast.***
Sl. 2: *T. marmorata****, ujet v obrežnih vodah Languedoca.***

Torpedo nobiliana Bonaparte, 1835 (Fig. 3): black torpedo (En), torpille noire (Fr)

Torpedo nobiliana is widely distributed and reported on both sides of the Atlantic (Bigelow & Schroeder, 1953). In the Mediterranean, it occurred off the coasts of its eastern basin (Capapé & Desoutter, 1980), in its eastern basin off Greece (Economidis, 1973) and Turkey (Kabasakal, 2002), whereas the species' easternmost border has been the Levantine basin (Golani, 1996, 2005).

Off the Languedocian coast, *T. nobiliana* was first recorded by Euzet (1960). Between 1952 and 1961, Quignard *et al.* (1962) recorded 29 specimens captured by trawlers especially in winter, at 100 m depth, off Sète. The total length ranged between 280 and 840 mm, the largest male and the largest female were 665 mm and 840 mm long, respectively.

From 1988 to 2005, observations at landing sites of the area allowed to collect 8 specimens, 4 males and 4 females. The males ranged from 170 to 755 mm in total length and weighed from 150 to 4820 g, three were juvenile and one adult. The females ranged from 350 to 1,020 mm total length and weighed from 1,200 to 20,000 g, a single specimen was juvenile, the three others were adult (see Capapé *et al.*, 2006a, Tab. 2, records 14, 16 and 19). The last specimen observed in the area was captured in 2002 (Capapé *et al.*, 2006a).

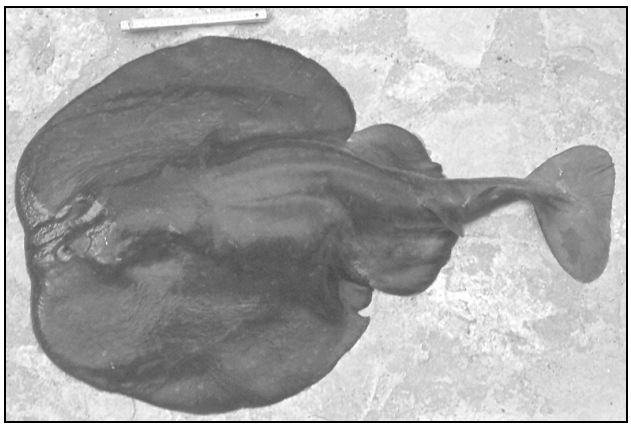

Fig. 3: *T. nobiliana* ***captured off the Languedocian coast.***
Sl. 3: *T. nobiliana****, ujet v obrežnih vodah Languedoca.***

Torpedo torpedo (Linnaeus, 1758) (Figs.4, 5): common torpedo (En), torpille ocellée (Fr)

The common torpedo is reported in the eastern Atlantic from the Bay of Biscay to Angola and it is commonly landed in tropical areas (Séret & Opic, 1990), especially off the coast of Senegal (Capapé *et al.*, 2000a). The species is recorded in the entire Mediterranean and

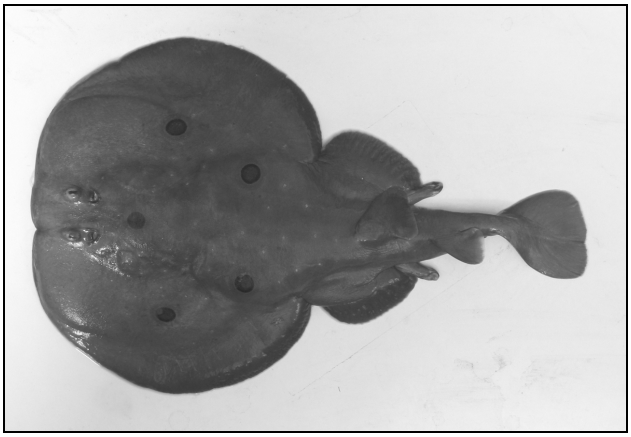

Fig. 4: Normal** T. torpedo **with five ocellae captured off the Languedocian coast (see Capapé** et al.**, 2006b).
Sl. 4: Normalen električni skat** T. torpedo **s petimi "očesnimi pegami", ujet v obrežnih vodah Languedoca (glej Capapé** et al.**, 2006b).

Fig. 5: Unusual** T. torpedo **with eight ocellae captured off the Languedocian coast.
Sl. 5: Nenavaden električni skat** T. torpedo **z osmimi "očesnimi pegami", ujet v obrežnih vodah Languedoca.

absent in the Black Sea (Capapé, 1989). Specimens from the Gulf of Tunis were studied by Quignard & Capapé (1974).

Off the Languedocian coast, the species was reported by Doumet (1860), Moreau (1881), Calvet (1905), Euzet (1959), Quignard *et al.* (1962) and Capapé *et al.* (2006b).

The common torpedo is rather rare in the area. Euzet (1959) recorded a single specimen, Quignard *et al.* (1962) 10 specimens, 2 males and 8 females between 1953 and 1961, ranging in total length between 140 and 365 mm. A female having 350 mm exhibited 7 ocellae on its dorsal surface.

Observations made at landing sites showed that all the specimens were caught by gill-netters, in shallow coastal waters, 20 m max. depth, on sandy bottoms. Twelve large specimens were recorded; of the 8 females, 2 were pregnant. Specimens of our sample were between 250 and 390 mm total length and weighed between 300 and 1,012 g, respectively. Capapé *et al.* (2006b) described 2 females exhibiting 6 and 9 ocellae, respectively, on their dorsal surface. The figure 5, included herein, shows a female, 305 mm in total length, with 8 ocellae on the dorsal surface.

Family Rajidae

Dipturus batis (Linnaeus, 1758): skate (En), pocheteau gris (Fr)

Dipturus batis is widely distributed in the northeastern Atlantic, from Scandinavia to northern Morocco (Muus & Dahlstrøm, 1964–1966), including the British Isles and, further south, the Strait of Gibraltar and the Island of Madeira (Blache *et al.*, 1970).

In the Mediterranean, *D. batis* is only known in the western basin, with the coast of Greece as its easternmost border (Economidis, 1973). Off the Maghrebin shore, *D. batis* is reported off the Algerian coast, but Bradaï *et al.* (2004) did not report it from Tunisian waters.

D. batis was reported by Doumet (1860), Moreau (1881), Calvet (1905), Euzet (1959), Granier (1964) and Quignard (1965) off the coast of Languedoc, where it was previously commonly recorded. No specimen was recorded since Quignard (1965).

Dipturus oxyrinchus (Linnaeus, 1758) (Fig. 6): longnosed skate (En), pocheteau noir (Fr)

Dipturus oxyrinchus was reported off Scandinavia (Muus & Dahlstrøm, 1964–1966), British Isles (Wheeler, 1969), the Bay of Biscay and off Portugal (Albuquerque, 1954–1956). South of the Strait of Gibraltar, *D. oxyrinchus* was reported around Madeira (Blache *et al.*, 1970).

D. oxyrinchus was reported throughout the Mediterranean, but captures were rather rare.

D. oxyrinchus was reported by Doumet (1860), Moreau (1881), Calvet (1905), Euzet (1959), Granier (1964) and Quignard (1965) off the coast of Languedoc, formerly commonly recorded in the area. No specimen was recorded since Quignard (1965). A single specimen was captured on 23 June 2006 by trawling, between Sète and Palavas, at depths between 80 and 100 m concomitantly with small spotted catsharks (see Fig. 1). It was a juvenile female having 345 mm in disc width and 480 mm in total length, and weighing 443 g.

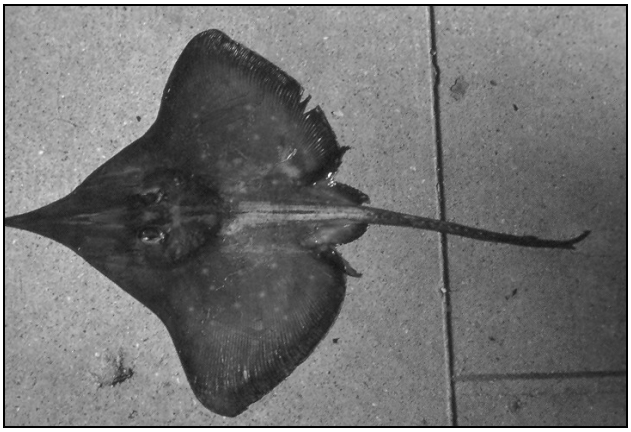

Fig. 6: *D. oxyrinchus* ***captured off the Languedocian coast.***
Sl. 6: *D. oxyrinchus****, ujet v obrežnih vodah Languedoca.***

Leucoraja circularis (Couch, 1838): sandy ray (En), raie circulaire (Fr)

Leucoraja circularis was reported off eastern Atlantic from Scandinavia to the northern coast of Morocco (Muus & Dahlstrøm, 1964–1966).

In the Mediterranean, the sandy ray was reported only in the western basin, its easternmost border being the northern coast of Greece (Economidis, 1973), and southernmost border the coast of Tunisia (Quignard & Capapé, 1971).

Moreau (1881) first reported the species off the coast of Languedoc, then by Quignard (1965) who considered it rather common in the area. No specimen has been found since Quignard (1965).

Leucoraja neavus (Müller & Henlé, 1841): cuckoo ray (En), raie fleurie (Fr)

Leucoraja neavus presented a distribution similar to that of *L. circularis*, in both the eastern Atlantic and the Mediterranean.

Off the Languedocian coast, the species was first recorded by Euzet (1959), while Quignard (1965) considered the species as commonly recorded in the area. No specimen has been found since Quignard (1965).

Raja asterias Delaroche, 1809 (Fig. 7): starry ray (En), raie étoilée (Fr)

The starry ray was reported throughout the Mediterranean, where it is rather common in northern areas, and in the western basin rather than in the eastern. Off the Tunisian coasts, for instance, *Raja asterias* was only recorded from the Algerian border to Bizerte, but is unknown further south.

R. asterias was successively reported off the Languedocian coast since Doumet (1860) to Quignard (1965). The authors considered it very common in the area. *R. asterias* was captured by trawlers and gill-netters throughout the year, although not abundantly. Preliminary observations were carried out on 51 specimens, 25 males and 26 females. Size of males ranged between 220 and 390 mm disc width, size of females between 220 and 480 mm disc width. All observed males and females were adult over 330 mm and 360 mm disc width, respectively. Reproductive activity of females occurred throughout the year, but it was difficult to show in which seas. Measurements made on 12 egg cases gave the following data: length with horns between 9.6 and 105 mm (mean: 10.2 mm ± 1.1), length without horns between 43 and 47 mm (mean: 45.9 mm ± 1.4), width between 32 and 34 mm (mean: 33.2 mm ± 0.40) and they weighed between 9.2 and 9.7 g (mean: 9.48 g ± 0.5).

Fig. 7: *R. asterias****, male (left) and female (right), captured off the Languedocian coast.***
Sl. 7: *R. asterias****, samec (levo) in samica (desno), ujeta v obrežnih vodah Languedoca.***

Raja brachyura Lafont, 1873 (Fig. 8): blonde ray (En), raie lisse (Fr)

The blonde ray was reported in the eastern Atlantic from British Isles (Wheeler, 1969) to Portugal (Albuquerque, 1954–1956), south of the Strait of Gibraltar the species is reported off northern Morocco (Lloris & Rucabado, 1998). In the Mediterranean, *R. brachyura* was rarely recorded, with its distribution similar to those of *Leucoraja naevus* and *L. circularis* (see above).

Euzet (1959) recorded for the first time a single specimen off the Languedocian coast, while Quignard (1965) noted that the species was very rare in the area. A single specimen was recorded, was captured on 14 April 1992 by trawling, between Sète and Palavas, at depths between 80 and 100 m (see above, *D. oxyrinchus*). It was an adult male having 620 mm in disc width and 915 mm in total length, and weighing 5,450 g.

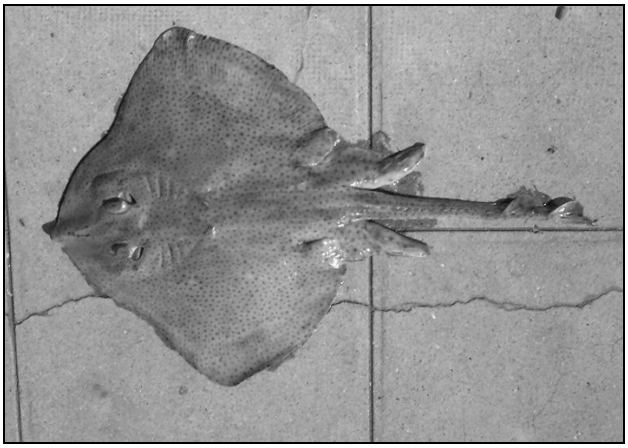

Fig. 8: *R. brachyura* ***captured off the Languedocian coast.***
Sl. 8: *R. brachyura* ***ujet v obrežnih vodah Languedoca.***

Raja clavata Linnaeus, 1758 (Fig. 9): thornback ray (En), raie bouclée (Fr)

The thornback ray presented a wide distribution in the Atlantic Ocean and the Mediterranean Sea. The species was reported in northern waters of the eastern Atlantic, from Scandinavia (Duncker, 1960), around British Islands (Wheeler, 1969) and Portugal (Albuquerque, 1954–1956). South of the Strait of Gibraltar, *R. clavata* was recorded off the Atlantic coast of Morocco (Collignon & Aloncle, 1972) and Mauritania (Maurin & Bonnet, 1970). The species was also recorded off the South African coast (Smith & Heemstra, 1986). The thornback ray is also reported from the Mediterranean (see Capapé, 1989) and entering the Black Sea according to Banarescu (1969) and Kabasakal (2002).

R. clavata was reported off the Languedocian coast from Doumet (1860) to Quignard (1965). The authors considered it very common in the area. A total of 257 specimens, 120 males and 137 females, were collected off the coast of Languedoc between 1988 and 2004. Samples were collected by gill-netters and trawlers at depths of up to 80 m, on sandy and muddy bottoms (see Fig. 1). They were generally landed in the harbours of Palavas-Les-Flots and Sète. Our results were summarized as follows (see Capapé *et al.*, *in press*). The smallest male and female adults had 420 mm and 540 mm disc width (DW), respectively, and weighed 2,130 g and 4,950 g,, respectively. The largest male and the largest female were 510 mm and 690 mm DW, respectively, and weighed 4500 g and 5980 g, respectively. There was a significant relationship total mass *versus* DW between males and females. Diameter of the largest yolky oocytes ranged from 24 to 27 mm (mean = 25.9 ± 0.9) and weighed from 3.2 to 3.7 g (mean = 3.5 ± 0.2). Vitellogenic activity occurred practically all year round, slightly less in April and August. Production of egg cases was observed throughout the year, except in April and August. Egg cases were between 122 and 127 mm (mean: 124.1 mm ± 1.3) in length with horns, and between 61 and 66 mm (mean: 63.6 mm ± 1.4) in length without horns, with their width between 50 and 56 mm (mean: 52.9 mm ± 1.5), and weighed between 19.5 and 22.5 g (mean: 20.9 g ± 1.4). Fecundity remained difficult to assess, an estimation based on production of egg cases and/or number of yellow yolky oocytes during the year counted in adult females, enabled us to consider it between 108 and 262.

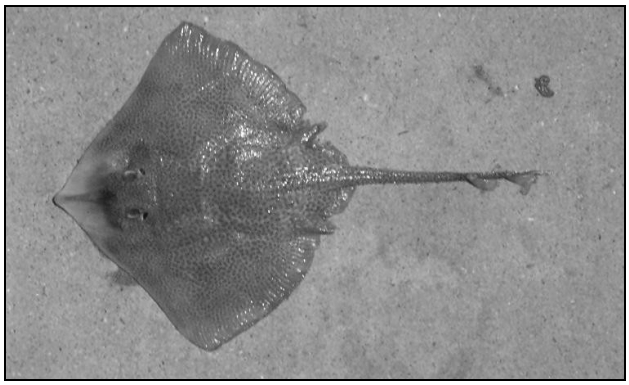

Fig. 9: *R. clavata* ***captured off the Languedocian coast.***
Sl. 9: *R. clavata****, ujeta v obrežnih vodah Languedoca.***

Raja miraletus Linnaeus, 1758: brown ray (En), raie miroir (Fr)

North of the Strait of Gibraltar, *Raja miraletus* was reported off Portugal (Albuquerque, 1954–1956), southwards from Morocco to South Africa (Séret & Opic, 1990). Off Senegal, *R. miraletus* is abundantly captured throughout the year in shallow coastal waters. The species is not locally used for human consumption as fresh or dried and is of no commercial interest, so specimens were generally discarded at sea by fishermen after their capture (Capapé *et al.*, 1995). The brown ray is found in the Mediterranean, where it is commonly caught off the southern shore and in the eastern basin (Capapé & Quignard, 1974; Golani, 1996, 2005).

R. miraletus was reported off the Languedocian coast from Doumet (1860) to Quignard (1965). Euzet (1959) and Quignard (1965) recorded only few specimens and considered the occurrence of the species very rare in the area. In contrast, Granier (1964) noted that the species was commonly reached at about 1,300 mm in total length; this measurement was no doubt excessive, as it did not exceed 600 mm according to literature. No specimen was recorded in the area since Quignard (1965).

Raja montagui Fowler, 1810: spotted ray (En), raie douce (Fr)

Raja montagui was considered relatively common in the eastern Atlantic, especially off the British Isles (Wheeler, 1969) and France (Du Buit, 1974). According to Du Buit (1974), the spotted ray occurred in the Bay of Biscay, while Lozano Rey (1928) reported it off Spain and Albuquerque (1954–1956), off Portugal. South of the Strait of Gibraltar, *R. montagui* was reported off Morocco (Lloris & Rucabado, 1998), with Mauritania as its southernmost border according to Maurin & Bonnet (1970).

In the Mediterranean, Tortonese (1956) and Bini (1967) reported the species from Italian waters, Šoljan (1963) from the Adriatic, Economidis (1973) from Greece, and Golani (2005) from waters off Israel.

The spotted ray was reported off the Languedocian coast by Euzet (1959), and, to date, no new records have been made in the area.

Raja polystigma Regan, 1923 (Fig. 10): speckled ray (En), raie tâchetée (Fr)

Raja polystigma is probably endemic to the Mediterranean Sea (Capapé, 1989), and was reported from some areas such as the Catalan Sea (Matallanas, 1977), off Toulon (southern France (Capapé, 1977), Italian seas (Tortonese, 1956; Arbocco, 1966), off Greece (Economidis, 1973; Kaspiris, 1974), Algeria (Dieuzeide *et al.*, 1953) and the Tunisian coast (Capapé & Quignard, 1978; Capapé *et al.*, 1980; Bradaï *et al.*, 2004).

Off the Languedocian coast, *R. polystigma* was only reported by Quignard (1965), however, no specimen was available for confirmation. The specimen described by Capapé *et al.* (2006b) confirmed the occurrence of the species in the area.

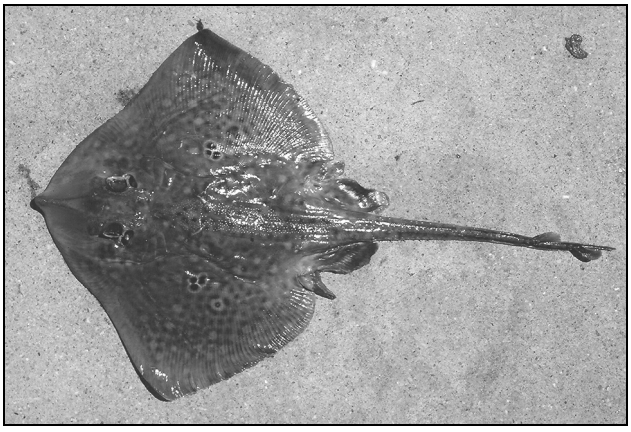

Fig. 10: *R. polystigma* ***captured off the Languedocian coast (see Capapé*** *et al.****, 2006c).***
Sl. 10: *R. polystigma****, ujeta v obrežnih vodah Languedoca (glej Capapé*** *et al.****, 2006c).***

Raja undulata Lacépède, 1802 (Fig. 11): undulate ray (En), raie brunette (Fr)

The undulate ray was found in the eastern Atlantic, south of the British Isles (Wheeler, 1969), off France (Du Buit, 1974), Spain (Lozano Rey, 1928) and Portugal (Albuquerque, 1945–1956). South of the Strait of Gibraltar, *Raja undulata* was known to occur off Morocco, and Mauritania could be its southernmost border according to Maurin & Bonnet (1970).

In the Mediterranean, *R. undulata* is reported in the western basin, off Spain (Lozano Rey, 1928), Italian seas (Tortonese, 1956; Bini, 1967) and in the Adriatic (Šoljan, 1963) down to Greece (Economidis, 1973). The species was recorded off Israel by Golani (2005).

R. undulata was reported off the Languedocian coast from Doumet (1860) to Quignard (1965), where it was considered rather common.

Two adult specimens were recorded, captured on 25 May 1992 by trawler between Sète and Palavas at depths between 80 and 100 m: one male and one female having 322 mm and 386 mm in disc width, 484 mm and 580 mm in total length, respectively, and weighing 765 g and 1,356 g, respectively.

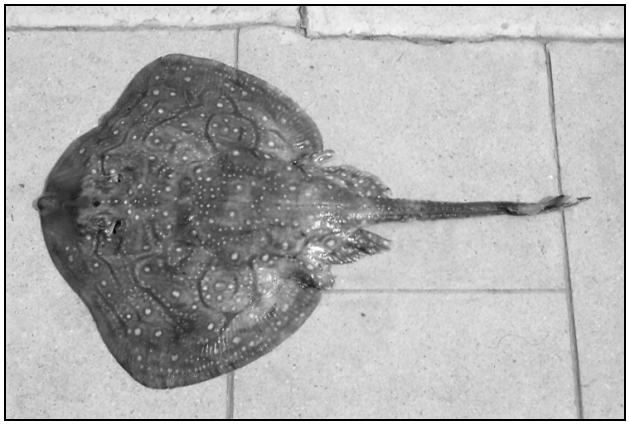

Fig. 11: *R. undulata* ***captured off the Languedocian coast.***
Sl. 11: *R. undulata****, ujeta v obrežnih vodah Languedoca.***

Raja rondeleti Bougis, 1959: Rondelet's ray (En), raie de Rondelet (Fr)

Bougis (1959a) showed that *Raja rondeleti* (= *Raja fullonica sensu* Rondelet) and *Leucoraja fullonica*, Linnaeus, 1758 were relatively close but distinct species. The Mediterranean occurrence of *L. fullonica* has never been proved; the description by Tortonese (1956), for instance, was based on one specimen from the Museum of Livourne, assigned by Bougis (1959a) to *R. rondeleti*. Dieuzeide & Novella (1952) noted that the species was very rare off Algeria, moreover, Roland (1952) added

that he had never recorded the species in the area. Wheeler (1969) observed *L. fullonica* off the British Isles, reporting on its restricted occurrence in the eastern Atlantic. Quignard (1965) considered *R. rondeleti* very rare off the Languedocian coast, where no record has been made since Bougis (1959a).

Two close but probably distinct species occurred in two different areas: *L. fullonica* in the eastern Atlantic, north of the Strait of Gibraltar, and *R. rondeleti* in some Mediterranean areas, such as the Languedocian coast and the Gulf of Genes.

Rostroraja alba (Lacépède, 1803): white skate (En), pocheteau blanc (Fr)

The white skate is known to occur in the eastern Atlantic south of Ireland and British Isles (Wheeler, 1969), off France (Du Buit, 1974), Spain (Lozano Rey, 1928) and Portugal (Albuquerque, 1954–1956). South of the Strait of Gibraltar, *Rostroraja alba* was reported off Morocco (Collignon & Aloncle, 1972) and Mauritania (Maurin & Bonnet, 1970). Cadenat (1950) reported the species off Senegal, but was not recorded there by Séret & Opic (1990) and Capapé *et al.* (1995). It remains absent in the Gulf of Guinea, but was sited further south, off South Africa, and probably occurred in the Indian Ocean off the southern part of Africa (Smith & Heemstra, 1988).

In the Mediterranean, the sandy ray was only reported in the western basin, its easternmost border being the northern coast of Greece (Economidis, 1793), and its southernmost border the coast of Tunisia (Quignard & Capapé, 1971).

R. alba was reported off the Languedocian coast from Doumet (1960) to Quignard (1965). No specimen has been recorded in the area since Quignard (1965).

Dasyatis pastinaca (Linnaeus, 1758): common stingray (En), pastenague commune (Fr)

The common stingray had a wide distribution in the eastern Atlantic from the North Sea (Muus & Dahlstrøm, 1964–1966) to Mauritania (Maurin & Bonnet, 1970). Moreover, the species has not occurred off Senegal, where its close relative, the marbled stingray, *Dasyatis chrysonota*, was abundantly caught according to Capapé *et al.* (1995).

D. pastinaca was reported throughout the Mediterranean and entering the Black Sea (Banarescu, 1969; Kabasakal, 2002).

D. pastinaca was reported off the Languedocian coast from Doumet (1860) to Quignard *et al.* (1962). Information provided by fishermen suggests that the species could occur in the area, although it has not been recorded there as yet.

Dasyatis violacea Bonaparte, 1832 (Fig. 12): pelagic stingray (En), pastenague violette (Fr)

According to Mollet (2002), the pelagic stingray is a species with a wide cosmopolitan distribution in several oceans and seas.

D. violacea is known in several areas of the Mediterranean, especially along the Maghrebin shore (Hemida *et al.*, 2003) and Italian coasts (Orsini Relini *et al.*, 2002), in the Adriatic Sea (Mavrič *et al.*, 2004) and off Israel (Golani, 2005).

D. violacea was reported from Doumet to Quignard *et al.* (1962). Some specimens were caught in the area, which allowed us to include them in the study of its reproductive biology based on specimens collected from three Mediterranean areas (Hemida *et al.*, 2003).

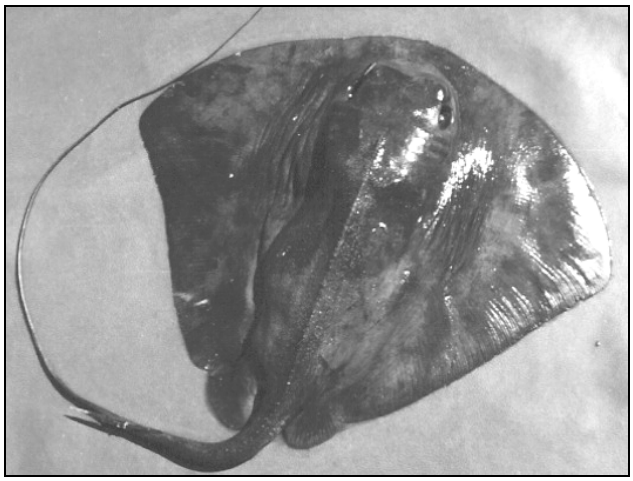

Fig. 12: *D. violacea* ***captured off the Languedocian coast.***
Sl. 12: *D. violacea****, ujet v obrežnih vodah Languedoca.***

Myliobatis aquila (Linnaeus, 1758) (Fig. 13): common eagle ray (En), aigle de mer commun (Fr)

The common eagle ray was reported in the eastern Atlantic from Scandinavia (Muus & Dahltstrøm, 1964–1966), off the British Isles (Wheeler, 1969), off France (Bougis, 1959b) and off Portugal, where recorded by Albuquerque (1954–1956). South of the Strait of Gibraltar, Postel (1959) reported on *Myliobatis aquila* from Cape Spartel to Cape Toxo, Collignon & Aloncle (1972) off Morocco and Maurin & Bonnet (1970) off Mauritania. Cadenat (1950) noted its occurrence off Senegal, but no specimens were observed from 1950 to date. In contrast, its close relative, the bull ray, *Pteromylaeus bovinus* (E. Geoffroy Saint-Hilaire, 1817), was rather abundant according to Seck *et al.* (2002) in the area. Sanchès (1991) registered *M. aquila* off Guinea-Bissau, which we are considering as its southernmost border. Its occurrence further south remains questionable.

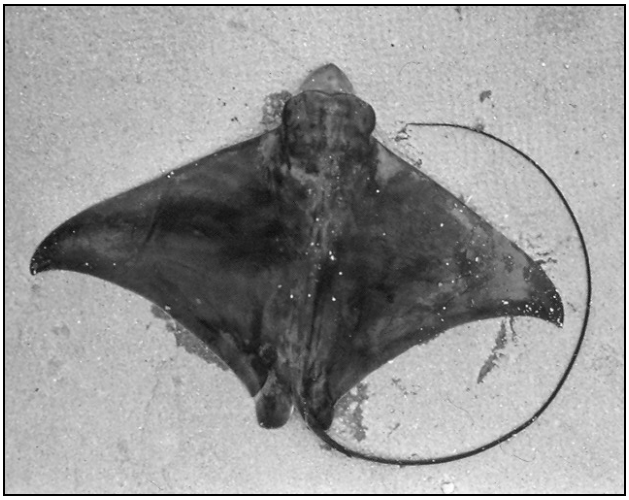

Fig. 13: *M. aquila* ***captured off the Languedocian coast.***
Sl. 13: *M. aquila****, ujet v obrežnih vodah Languedoca.***

The common eagle ray was reported throughout the Mediterranean and it was abundantly caught in Tunisian waters (Capapé & Quignard, 1974; Capapé, 1989).

M. aquila was reported off the Languedocian coast from Doumet (1860) to Quignard *et al.* (1962). Captures made in the area between 1990 and 2004 allowed us to present some traits of its reproductive biology, summarized below as unpublished data. In all, 73 common eagle rays were observed, 41 males and 32 females. They were captured by demersal gill-nets at depths from ca. 30 to 40 m between Sète and Le Grau-du-Roi, from 1990 to 2004. The smallest male and female adults had 500 mm and 730 mm disc width, respectively, and weighed 2,000 g and 6,100 g. The largest male and the largest female had 720 mm and 1,140 mm disc width, respectively, and weighed 5,250 g and 29,400 g, respectively. There was a significant relationship total mass *versus* disc width between males and females. Diameter of the largest yolky oocytes ranged from 26 to 32 mm, number of yolky oocytes counted in five females ranged between 8 and 10. Fertilized eggs were enveloped in a diaphanous capsule. Each capsule contained six eggs weighing between 4.5 and 5.3 g (mean: 4.9 ± 0.9). The breeding period probably lasted from August to September. The common eagle ray possibly reproduced in alternate year, with the length of embryonic development not exceeding one year. Ovarian fecundity and uterine fecundity were rather low, both between 8 and 12.

Tab. 1: List of the 22 skates and rays reported from Doumet (1860) to date off the Languedocian coast.
Tab. 1: Seznam 22 vrst skatov iz obrežnih voda Languedoca, o katerih so poročali od Doumeta (1860) dalje.

Species	Doumet (1860)	Moreau (1881)	Calvet (1905)	Euzet (1959)	Quignard *et al.* (1962)	Granier (1964)	Quignard (1965)	This study
Pristis pristis	–	+	–	–	–	+	–	–
Rhinobatos rhinobatos	–	–	–	–	–	+	–	–
Torpedo marmorata	+	+	+	+	+	+	–	+
T. nobiliana	–	–	–	+	+	–	+	+
T. torpedo	+	+	+	+	+	+	–	+
Dipturus batis	+	+	+	+	–	+	+	–
D. oxyrinchus	+	+	+	+	–	+	+	+
Leucoraja circularis	–	+	–	–	–	–	+	–
L. naevus	–	–	–	+	–	–	+	–
Raja asterias	+	+	+	+	–	+	+	+
R. brachyuran	–	–	–	+	–	–	+	+
R. clavata	+	+	+	+	–	+	+	+
R. miraletus	+	+	+	+	+	+	+	–
R. montagui	–	–	–	+	–	–	–	–
R. polystigma	–	–	–	–	–	–	+	+
R. undulate	+	+	+	+	+	+	+	+
R. rondeleti	–	–	–	–	–	–	+	–
Rostroraja alba	+	+	+	+	+	+	+	–
Dasyatis pastinaca	+	+	+	+	+	+	+	–
D. violacea	+	+	+	+	+	+	+	+
Myliobatis aquila	+	+	+	+	+	+	+	+
Mobula mobular	–	–	–	–	–	+	–	+

Family Mobulidae

Mobula mobular (Bonnaterre, 1788) (Fig. 14): devil ray (En), diable de mer (Fr)

The devil ray is defined as an Atlantic-Mediterranean species (Fischer *et al.*, 1981, 1987; Mc Eachran & Capapé, 1984; Celona, 2004). However, its occurrence in the eastern Atlantic waters remains hypothetical according to Notarbartolo di Sciarra & Bianchi (1998); misidentifications with the close related species *M. japonica* could not be occulted.

In the eastern Atlantic, a single capture of *M. mobular* was reported by Lozano Rey (1928) off Cadix. Moreover, south of the Strait of Gibraltar, *M. mobular* was not reported off Mauritania (Maigret & Ly, 1986) and off Guinea-Bissau (Sanchès, 1991). Off Senegal, several species of the genus *Mobula* were abundant and regularly caught during some periods of the year, but Cadenat (1960), among others, did not record *M. mobular* there. In contrast, Capapé *et al.* (1994, 1995) recorded some specimens off Oukam, fishing site located at Cape Verde Peninsula.

These considerations and recent captures of *M. mobular* in the central and eastern areas of the Maghrebin coast suggest endemicity of the species in the Mediterranean, which agrees with Notarbartolo Di Sciara & Bianchi (1998). However, migrations through the Strait of Gibraltar could not be excluded.

Formerly, the captures of giant devil ray off the Algerian coast were accidental and rather considered as ichthyological events (Dieuzeide & Novella, 1952). They generally concerned one or two exemplars. The specimens reported in this paper and information provided by fishermen suggest that the species is commonly caught in the area. Similar observations were made in Tunisian waters. The first specimen, a gravid female containing a fully developed foetus, was previously recorded by Capapé & Zaouali (1976) along the northern coast, off Sidi-Daoud., Between 1999 and 2000, however, Bradaï & Capapé (2001) reported the captures of five large specimens, in the Gulf of Gabès, southeastern Tunisia. So, captures of *M. mobular* were made in a restricted area, which extends from the central area of the Algerian coast to the Gulf of Gabès. In the Mediterranean, the recorded captures occurred in the western basin. They were made in winter off the southern coast, mostly in spring off the northern coasts. Captures of specimens of both sexes and of different sizes suggest trophic migrations through the area, but genesic migrations cannot be excluded (Hemida *et al.*, 2002).

Off the Languedocian coast, Granier (1964) observed four juvenile specimens having between 1.80 and 2 m in disc width, swimming off Le Grau-du-Roi. Capapé *et al.* (1990) observed one adult male with 2.20 m in disc width captured by gill-nets off Le Grau-du-Roi. No specimen has been captured to date.

Fig. 14: *M. mobular* ***captured off the Languedocian coast (see Capapé*** *et al.,* ***1990).***
Sl. 14: *M. mobular****, ujet v obrežnih vodah Languedoca (glej Capapé*** *et al.,* ***1990).***

DISCUSSION

A literature review based on historical and recent papers show that 22 skate and ray species were reported off the Languedocian coast between Doumet (1860) and this study according to Table 1. Of the 12 species recently recorded from Quignard *et al.* (1962) and Quignard (1965) to date, more than four decades ago, only five were regularly landed at the area's fishing sites (Tab. 2): *Torpedo marmorata*, *T. torpedo*, *Raja asterias*, *Dasyatis violacea* and *Myliobatis aquila*. *R. asterias* has a commercial value and is locally sought after for consummation, *M. aquila* slightly less, whereas the three other species are generally discarded at sea by fishermen. With special regard to the capture of *R. polystigma,* Capapé *et al.* (2006c) noted that it did not suggest a recovery of the species in the area, but it occurred in deep biotope previously unexploited by usual fishing methods according to information provided by fishermen. Other similar records cannot be excluded. *D. violacea* was formerly recorded more or less along the northern African shore, while the recent occurrence of the species in northern Mediterranean areas, such as the Tyrrhenian and Northern Adriatic Seas, could be related to the phenomena of tropicalisation.

A decline of skate and ray captures is the result of an intensive fishing pressure. As a consequence, formerly abundant species, such as the thornback ray and the speckled ray, are now considered rare exceptions off the Languedocian coast. Other abundant species, such as the brown ray, the cuckoo ray, the white ray and the common stingray, have completely disappeared from the area. Similar patterns were reported for sharks (see

Tab. 2: Previous and present status of the 12 skates and rays reported to date from the coastal waters of Languedoc.
Tab. 2: Prejšnji in današnji status 12 vrst skatov, zabeleženih vse do danes v obrežnih vodah francoske regije Languedoc.

Species	Last record	Previous status	Present status
Torpedo marmorata	2006	A	A
T. nobiliana	2002	R	R
T. torpedo	2006	C	C
Dipturus oxyrinchus	2006	C	R
Raja asterias	2006	A	A
R. brachyura	1992	C	R
R. clavata	2006	A	R
R. polystigma	2006	R	R
R. undulata	1992	C	R
Dasyatis violacea	2006	C	C
Myliobatis aquila	2006	C	C
Mobula mobular	1991	R	R

Capapé *et al.*, 2000b). Soldo (2003) noted that sharks are strong *K* selected species; this is also the case of skates and rays. Du Buit (1989) noted that landings of large rays, such as *Dipturus batis* and *Rostroraja alba*, were particularly important off the Atlantic coast of France. Captures of the former reached 863 t in 1965 and 75 t in 1986, while the latter has completely disappeared. Du Buit (1989) added that skates and rays are especially vulnerable with respect to their morphology: soon after hatching, the disc width of skate species is larger than the authorized mesh net size, and consequently the mortality of juveniles is very high, more than in other fish species, including sharks and teleosts. Unfortunately, no statistical data are available concerning especially skates and rays of the Languedocian coastal waters. The risk of extinction of some species in the area cannot be excluded. Moreover, skates and rays are not considered in the FAO status evaluation. Consequently, it appears that skates and rays could not present a stable biomass at any sites of the area. *T. marmorata* and *T. torpedo* constitute two exceptions, probably because they inhabit shallow coastal waters, sometimes less than one metre deep, where they are rarely fished. They entered protected areas, such as some brackish lagoons along the Languedocian shore (Paris & Quignard, 1971). Moreover, they have no commercial value and are discarded, generally alive, by fishermen when caught.

SKATI (CHONDRICHTHYES) V OBREŽNIH VODAH LANGUEDOCA (JUŽNA FRANCIJA, SEVERNO SREDOZEMLJE): ZGODOVINSKI PREGLED IN NJIHOV SEDANJI STATUS

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POVZETEK

Pregled ihtioloških člankov, objavljenih med letoma 1860 in 1965, kaže, da je bilo v tem obdobju v vodah francoske regije Languedoc (južna Francija, severno Sredozemlje) zabeleženih 22 vrst iz reda skatov. Raziskave, ki so potekale od leta 1988 dalje, pa kažejo, da je bilo v tem območju opaženih samo 12 vrst, 2 od katerih bi lahko opisali kot številne, 3 pa razmeroma pogoste. Druge vrste so se le tu in tam ujele v mreže lokalnih ribičev, kar gre bržkone pripisati pretiranemu izlovu teh hrustančnic v preteklosti.

Ključne besede: Chondrichthyes, skati, obalne vode Languedoca, Sredozemlje

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ON THE OCCURRENCE OF RIBBON FISH *TRACHIPTERUS TRACHYPTERUS* (GMELIN, 1789) IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA)

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ABSTRACT

Five adult specimens of ribbon fish Trachipterus trachypterus *were registered in the Gulf of Trieste during the summer of 2006. The species is widely distributed, but the records in this area are rare. The morphometric and meristic characteristics and the stomach contents of four specimens are described. Comparison with other reports of rare species in the same area and evolution of the thermohaline properties in the Gulf of Trieste are also considered.*

Key words: *Trachipterus trachypterus*, stomach contents, rare species, Gulf of Trieste, Adriatic Sea

SEGNALAZIONI SULLA COMPARSA DEL PESCE NASTRO *TRACHIPTERUS TRACHYPTERUS* (GMELIN, 1789) NEL GOLFO DI TRIESTE (ADRIATICO SETTENTRIONALE)

SINTESI

Cinque esemplari adulti di pesce nastro Trachipterus trachypterus *sono stati ritrovati nel Golfo di Trieste durante l'estate 2006. La specie presenta un'ampia distribuzione geografica ma le segnalazioni in queste acque sono rare. Vengono riportate le caratteristiche morfometriche e meristiche e vengono descritti i contenuti stomacali di quattro esemplari. I ritrovamenti vengono confrontati con altre segnalazioni di specie rare nella stessa area e vengono evidenziate alcune relazioni con le caratteristiche termoaline del Golfo di Trieste.*

Parole chiave: *Trachipterus trachypterus*, contenuti stomacali, specie rare, Golfo di Trieste, Mare Adriatico

INTRODUCTION

The ribbon fish *Trachipterus trachypterus* (Gmelin, 1789) is a meso-pelagic species, resident primarily between 200 and 1000 m. It inhabits tropical and subtropical waters of every ocean, occurring in central Pacific Ocean, Japan, New Zealand, south-eastern coasts of Africa and Mediterranean Sea (except eastern Black Sea) (Fischer *et al.*, 1987; Jardas, 1996). Belon (1553) initially named the species *Falx venetorum*. In the Mediterranean, common names for this species exist in France ('poisson ruban'), Greece ('kordélla'), Israel ('trakhipterus'), Italy ('pesce nastro'), Slovenia ('kosica'), Croatia ('mač srebrnjak'), Malta ('fjamma'), Monaco ('pisciu rüban'), Spain ('lista') and Turkey ('kâğit') (Bini, 1969), but no dialectal names are known in the Adriatic Sea (Šoljan, 1975). It is occasionally caught by purse seines, trawling nets and long-lines (Fischer *et al.*, 1987). The species is sporadically recorded in all Italian seas, but only in the Messina Strait it occurs 1–10 times per year (Costa, 1991). Between 1875 and 1980, only 46 adult individuals were recorded in the Adriatic (Jardas, 1980).

More frequently, ribbon fishes are seen dead, floating on the surface or washed to the beach either by waves or tides. Their presence at the surface is probably not normal (Bini, 1969).

The data on biology and ecology of this species are very scarce. The aim of this paper is to provide new additional data on its occurrence in the Gulf of Trieste and on its distribution and biology in the Adriatic Sea.

MATERIAL AND METHODS

Between July and September 2006, five specimens of ribbon fish *Trachipterus trachypetrus* (Trachipteridae) were recorded in the Gulf of Trieste.

The first specimen was recorded on July 31, at a depth of 1.5 m, inside the little harbour of "Filtri di Aurisina", in front of the Department of Biological Oceanography of the National Institute of Oceanography and Applied Geophysics (OGS) of Trieste (Fig. 1). In August, two adult specimens were caught off Izola (Slovenia): the first at the beginning of August by a pelagic trawling net, the second on August 10 by a monofilament gillnet, set about 2 NM from the coast (V. Žiža, *pers. comm.*). In September, two other adult specimens were registered: one on September 2, caught by hands at a depth of 0.5 m outside of the same little harbour where the first specimen was found; the other along the seaside of Barcola (Trieste) (N. Bressi, *pers. comm.*). The two adult specimens found at Aurisina were alive, moving slowly with one side turned obliquely.

A juvenile specimen of *T. trachypterus*, found on 15 April 2004 in the coastal waters of Piran (Slovenia), was used to compare the morphology between adult and juvenile stages. The specimen was dead and injured, lacking the caudal part. It was preserved in 10% buffered formalin at the laboratory of the Marine Biological Station Piran (National Institute of Biology).

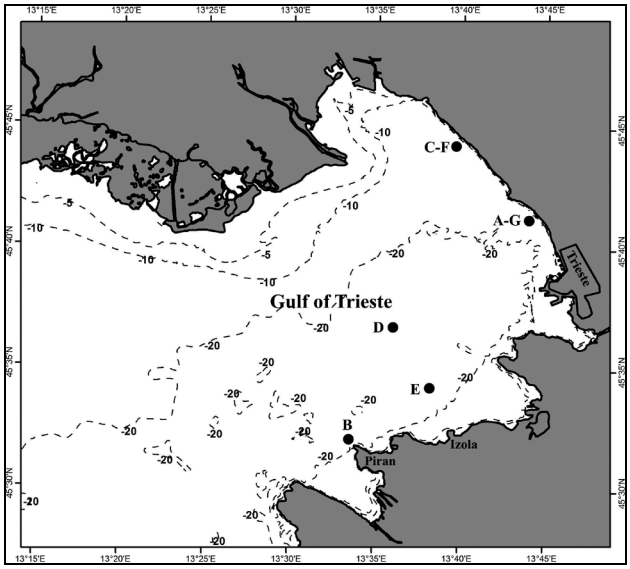

Fig. 1: Locations of** T. trachypterus **records in the Gulf of Trieste: A (05/08/03, Barcola), B (15/04/04, Piran), C (31/07/06, Aurisina Filtri), D (?/08/06, off Izola), E (10/08/06, 2 NM off Izola), F (02/09/06, Aurisina Filtri), G (?/09/06, Barcola).

Sl. 1: Lokacije v Tržaškem zalivu, na katerih je bila zabeležena kosica** T. trachypterus**: A (05/08/03, Barkovlje), B (15/04/04, Piran), C (31/07/06, Nabrežina Filtri), D (?/08/06, Izola), E (10/08/06, 2 NM stran od Izole), F (02/09/06, Nabrežina Filtri), G (?/09/06, Barkovlje).

In the laboratory, meristic (Tab. 1) and morphometric (Tab. 2) characteristics were described and the fishes accurately identified according to Bini (1969), Šoljan (1975), Fischer *et al.* (1987), Costa (1991) and Jardas (1996). Length measurements were taken to the nearest 1 mm and weight measurements to the nearest 0.1 g. The stomach of each fish was dissected under a stereomicroscope and the whole content was washed out on a petri dish and examined individually (at 70X magnification). Each prey item was counted and determined at the lowest possible taxonomical level. Comparisons with description of other specimens found in the Mediterranean were made.

RESULTS AND DISCUSSION

Morphology

This species possesses all the characteristics of fish living at very great depths. The body is elongated and laterally compressed. Largest height is immediately behind the short head. Colour is silvery, with 3 (sometimes 4) dark blotches over lateral line and 1 in ventral position,

near the head. Fins are red-orange, with a delicate connective membrane (Fig. 2). Dorsal fin is long, as the whole length of the back, with the first few rays elongated in juveniles, but often reduced in adults. Anal fin is absent. Pectoral fins are short and inserted horizontally. Ventral fins are inserted on the ventral profile, with 3–9 very elongated rays in juveniles, but small or absent in adults. Caudal fin consists of two fascicles of rays, the upper elongated and directed upwards, the lower rudimental and sub-horizontal. Frontal space is black. Eyes are big and circular (Fig. 3). Mouth is small and very protrusible. Teeth are subtile and projecting backwards. The skin is scaleless, but uniformly covered by little bony tubercles (only in adults). The lateral line is formed by bony plates, each with a spine projecting ahead (more evident in the caudal region).

The general shape of body and fins are greatly modified during maturation of the individuals. The body in juveniles is higher, but shorter, and the anterior profile of the head is more steep (Fig. 4). The eyes are located in the centre of the head in juveniles, while in adults they are situated near the dorsal profile.

In adults, some characters are drastically reduced. In young specimens, the first 5–6 dorsal fin rays and the ventral fin rays are extraordinarily long and provided with appendages. At the length of 10 cm, rays of the dorsal and ventral fins become shorter and the caudal fin become similar to a fan. In adults, the swimming bladder is heavily reduced and the teeth are less abundant.

Generally, they hold their body in vertical position, with head upwards and undulating their long dorsal fin. The juveniles use the elongated fins (dorsal, ventral and pectoral) like sails, drifting passively in the current.

Meristic characters (Tab. 1) of the analysed specimens are in agreement with data from specimens of the Adriatic Sea (Jardas, 1980) and other Italian seas (Bini, 1969). The differences recorded in the number of ventral fin rays and teeth in the upper jaw are due to the different life stage of the individuals analysed; in adults, in fact, these characters tend to be reduced.

The species reaches 300 cm (Tortonese, 1970; Šoljan, 1975), but the biggest specimen recorded for the Adriatic Sea was 160 cm long; it was caught in Bakar Bay (near Rijeka, Croatia) in 1951 (Jardas, 1980). Total length of the 33 individuals measured in the Adriatic Sea from 1888 to 1979 ranged between 13.7 and 160.0 cm, and 9 of them were more than 100 cm long (Jardas, 1980).

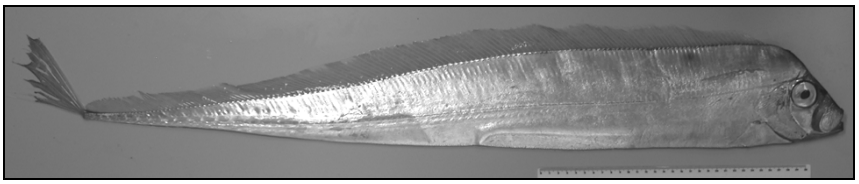

Fig. 2: Specimen of *T. trachypterus* ***caught on 31 July 2006 in front of the Institute for Biological Oceanography (OGS) of Trieste (30 cm barr is visible right down).***
Sl. 2: Osebek vrste *T. trachypterus,* ***ujet 31. julija 2006 pred tržaškim Inštitutom za biološko oceanografijo (OGS) (30 cm merilo v desnem spodnjem kotu).***

Tab. 1: Meristic characters in each of the specimens recorded recently in the Gulf of Trieste compared with those recorded by other authors (* referred to juveniles; n.d. not determinable).
Tab. 1: Meristični podatki osebkov, nedavno zabeleženih v Tržaškem zalivu, primerjani s podatki drugih avtorjev (*mladostni osebki; n.d. nedeterminirani).

Meristic characters	Specimen				Author	
	15/04/04	31/07/06	10/08/06	02/09/06	Bini (1969)	Jardas (1980)
dorsal fin rays D	n.d.	160	180	165	125–187	126–181
ventral fin rays V	5	3	0	0	8*	5–7*
pectoral fin rays P	10	10	11	10	11	9–12
caudal fin rays (upper lobe)	n.d.	8	9	7	6–8	7–8
caudal fin rays (inferior lobe)	n.d.	6	5	6	3–6	
spines along lateral line	n.d.	97	94	91		
right lower jaw (dental) teeth	5*	5	5	5	4–5*	
left lower jaw (dental) teeth	5*	4	4	5	4–5*	
vomer teeth	1*	1	1	1	some*	
right upper jaw (praemaxillary) teeth	7*	7	5	3	8–10*	
left upper jaw (praemaxillary) teeth	4*	5	4	2	8–10*	
gillrakers (1st branchial arch)	12*	13	13	13		

Tab. 2: Morphometric characters and organ weights in the specimens recorded in the Gulf of Trieste (n.d. not determinable).
Tab. 2: Morfometrični podatki in teža organov osebkov, zabeleženih v Tržaškem zalivu (n.d. nedeterminirani).

Date	**15/04/04**	**31/07/06**	**10/08/06**	**02/09/06**
Preservation	formol	frozen	formol	frozen
Morphometric characters (mm)				
total length (TL)	n.d.	998	1033	1205
standard length (SL)	n.d.	878	925	1067
preorbital length	5	26	23	39
eye horizontal diameter	9	32	35	36
head length (C)	28	97	98	111
head height	37	107	105	124
interorbital space	6	21	22	24
predorsal length (LPD)	7	51	55	68
prepectoral length (LPP)	29	93	99	108
preventral length (LPV)	33	105	111	118
maximal body height	39	108	108	126
preanal length (LPA)	110	400	405	500
caudal peduncle height	n.d.	10	11	12
dorsal fin rays maximal length	29	55	68	76
dorsal fin length	n.d.	835	882	990
pectoral fin length	7	26	33	42
caudal fin length (upper lobe)	n.d.	120	125	137
caudal fin length (lower lobe)	n.d.	0.8	1.2	2
ventral fin length	46	0	0	0
sex	n.d.	male	male	male
Weight (g)				
total weight	n.d.	495.2	477.7	1036.3
gutted weight	n.d.	411.7	406.8	956.0
heart	n.d.	0.6	0.8	0.8
stomach	1.4	11.6	22.5	27.1
empty stomach	0.6	7.3	20.9	21.1
liver	n.d.	2.7	6.8	10.3
piloric ceaca	n.d.	4.7	8.0	10.3
intestine	n.d.	1.6	2.6	5.6
gonads	n.d.	0.9	1.1	1.6

Diet

Data about the diet of this species are scarce. Bini (1969) and Tortonese (1970) consider the diet exclusively carnivore, including cephalopods, shrimps, pelagic and benthic fishes, or, rather, mid-water fishes (Dulčić & Lipej, 1997) and bathypelagic fishes (Costa, 1991).

In the stomachs of ribbon fishes caught in the Gulf of Trieste, both animal and vegetal organisms were found (Tab. 3). Those of adult specimens contained rests of teleost fishes, fragments of algae, marine phanerogams and earth-plants (Fig. 5). The stomach of the juvenile individual presented a wider diet spectrum, comprising numerous Copepods. Probably the vegetal fragments of earth-plants were accidentally ingested.

Jardas (1980) analysed the stomach content of 4 specimens and in 2 of them found *Cymodocea nodosa*, algal fragments of *Dilophus fasciela*, *Cystoseira fimbriata*, leaves of *Pinus* sp. and pieces of other earth-plants. He also found remains of polychaetes and other smaller shrimps, *Enteromorpha intestinalis*, *Coccinella septempunctata*, the cigarette filter and pieces of various plastic objects. The diversity of ingested objects indicates that the species is a voracious predator, eating virtually everything when missing its natural prey. According to the same author, this also indicates that the collected individuals were active for a certain period in the littoral region, though the area does not represent their natural environment.

Tab. 3: List of prey items found in the stomachs of** T. trachypterus **from the Gulf of Trieste.
Tab. 3: Seznam enot plena, najdenega v želodcih kosice** T. trachypterus **iz Tržaškega zaliva.

Prey items		Specimens			
group	**species**	**15/04/04**	**31/07/06**	**02/09/06**	**10/08/06**
Diatoms	*Coscinodiscus* sp.	1			
Dinoflagellates	*Protoperidinium* sp.	1			
Algae	*Cladophora dalmatica*		fragments		
	Rhizoclonium riparium		fragments		
Marine Phanerogams	*Cymodocea nodosa*			leaves and rhizome	leave
Earth-plants	*Cupressus cupressus*			leaves	
	Betula pendula	1 seed			
Cladocerans	*Evadne nordmanni*	1			
Copepods	*Acartia clausi*	1			
	Temora longicornis	8			
	Temora stylifera	2			
	Diaixis pigmoea	1			
	Calanus helgolandicus	1			
	Calanidae	9			
	Clauso-Paracalanidae	19			
	Oithona plumifera	23			
	Oithona nana	1			
	Corycaeus sp.	5			
	Euterpina acutifrons	5			
	Microsetella rosea	2			
	Copepoda nauplius	1			
Isopods	Isopoda	3			
Decapods	Alpheidae	1			
Bony fishes	*Engraulis encrasicolus*			1	
	Teleostea	3			

Parasites

On the skin of the specimen caught on 31 July 2006, 32 individuals of *Paragnathia formica* (Isopoda) and 2 parasitic Copepods of the genus *Caligus* (Cyclopoida) were counted.

Reproduction

Dulčić (1996) found, for the first time in the Adriatic, a larval specimen of *T. trachypterus* at Stončica near Vis Island (southern Adriatic). Bini (1969) reports that eggs and larvae of this species are found in the plankton of Messina Strait from November to May, suggesting that the spawning period extends to the whole year. On the beaches of the same strait, juvenile and adult specimens are generally recorded in March, April and May (Costa, 1991). Jardas (1980) observed that its higher frequency along the eastern Adriatic coasts during spring and summer months is correlated with the spawning period. All the adult specimens analysed in this work were males.

Fig. 3: Detail of head in adult** T. trachypterus**.
Sl. 3: Detajl glave odrasle kosice** T. trachypterus**.

Tab. 4: Date, location, length and reference of records of** T. trachypterus **in the Gulf of Trieste.
Tab. 4: Datum, lokacija, dolžina in vir kosic** T. trachypterus**, zabeleženih v Tržaškem zalivu.

Date	Locality	Length (cm)	Reference
1888	Gulf of Trieste	adult	Marcuzzi (1972)
1888	Gulf of Trieste	juvenile	Marcuzzi (1972)
?	Grignano	108	Jardas (1980)
?	Grignano	83	Jardas (1980)
?	Gulf of Trieste	67	Jardas (1980)
?	Gulf of Trieste	75	Jardas (1980)
02/1992	Ronek cape	110	Dulčić & Lipej (1997)
1992	Grignano	114	Bussani (1992)
05/08/2003	Barcola	105	R. Auriemma *(pers. comm.)*
15/04/2004	Piran	~25	this work
31/07/2006	Aurisina	100	this work
~01/08/2006	Gulf of Trieste	adult	V. Žiža *(pers. comm.)*
10/08/2006	2 NM off Izola	103	this work
02/09/2006	Aurisina	120	this work
09/2006	Barcola	110	N. Bressi (*pers. comm.*)

Comparison with other records

In the Mediterranean Sea, the occurrence of the species is rarely documented, although it is occasionally caught in the Aegean Sea (Papakonstantinou, 1988; Bilecenoglu *et al.*, 2002), Messina Strait (Costa, 1991) and the western Mediterranean (Tortonese, 1958; Fabre, 1967). Since the recorded specimens of *T. trachypterus* have been caught with different fishing gears, its rarity does not seem to be connected with the use of inappropriate fishing tecniques. This fact supports the belief that this species is truly rare.

In the Adriatic Sea, *T. trachypterus* was for the first time reported by Kolombatović (1881), but Faber (1883) described this species as generic, although rare, reporting it for Trieste, Venice and Dalmatia. Jardas (1980) reported on the capture of 46 specimens from the Adriatic Sea since 1875. Six of those individuals were no doubt from the Gulf of Trieste: 2 were recorded in 1888 and 4 between 1939 and 1951 (Tab. 4). There are no documented records for the 1951–1991 and 1993–2002 periods.

During the summer of 2006, i.e. in the same period when the records presented in this work were made, an adult specimen, about 1 meter long, was washed up by tide at Brussa (Caorle, Italy) on July 9 (A. Colla, *pers. comm.*). On 4 August 2006, another specimen of about 40 cm was caught at Dugi otok (central-eastern Adriatic) in the shallow waters of a little harbour by a fisherman using a harpoon (N. Burba, *pers. comm.*). Previous records had not been so frequent: on 15 April 2004, the juvenile specimen analysed in this work was registered at Piran, whereas on 5 August 2003, an adult specimen, 105 cm long, was found at the Barcola seaside (Trieste) (R. Auriemma, *pers. comm.*). In 1992, however, records of *T. trachypterus* were relatively common in the middle and northern parts of the Adriatic. In that year, sport-fishermen caught a 114 cm long specimen in the vicinity of Grignano harbour the Gulf of Trieste (Bussani, 1992).

According to Jardas & Pallaoro (1996), the occurrence of *T. trachypterus* in central and northern regions of the Adriatic might be associated with some special climatological and oceanographical conditions in 1992 and 1994, such as inputs of intermediate waters (50–100 m) from the eastern Mediterranean into the Adriatic, which influenced the increase in salinity and temperature (Buljan, 1953; Jardas, 1980; Vučetić, 1982). Pallaoro (1988) stated that the Adriatic ingressions in the 1986–87 period caused more rare species, such as *Centracanthus cirrus*, *Aulopus filamenosus*, *Pseudocharanx dentex*, *Synodus saurus*, *Centrolophus niger*, to appear in the central Adriatic region. The unusual abundance of such rarely found fish species compared to the non-ingression periods gives indication of their interdependence. Dulčić *et al.* (1999) found that most of new occurrences of fish species were recorded in the 1985–1987 and 1990–1995 periods, when 11 subtropical and tropical fishes were recorded for the first time.

Also in the Gulf of Trieste, which is the northernmost part of the Mediterranean Sea, thermohaline anomalies, occurring in spring-summer periods, have been related to advective flow of high salinity waters from the South into the basin (Celio *et al.*, 2002). For this region, the specific ichthyological list accounts as new species *Plectorhincus mediterraneus* (Lipej *et al.*, 1996), *Epinephelus marginatus* (Dulčić & Lipej, 1997), *Coryphaena hippurus* (Dulčić & Lipej, 1997), *Sphyraena sphyraena* (Žiža, 1997), *Ruvettus pretiosus* (Bettoso & Dulčić, 1999) and *Brama brama* (G. Barbieri, *pers. comm.*). It is interesting that in a less recent paper, Bus-

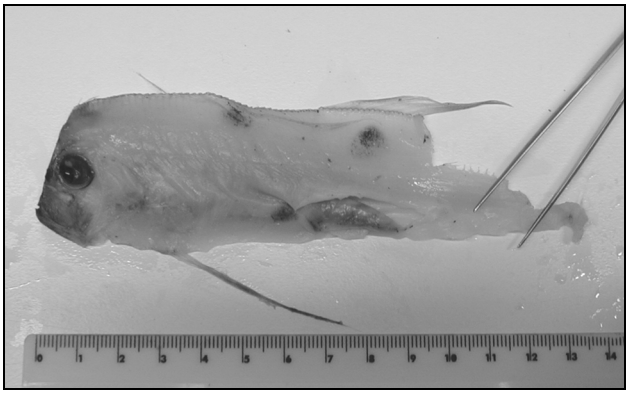

Fig. 4: Juvenile specimen found on 15 April 2004 off Piran.
Sl. 4: Mladostni osebek, najden 15. aprila 2004 v bližini Pirana.

Fig. 5: Stomach content of the specimen caught on 31 July 2006.
Sl. 5: Struktura hrane v želodcu osebka, ujetega 31. julija 2006.

sani & Feoli (1976) reported some other species rare for the Adriatic (especially for its northern part), such as *Centrolophus niger*, *Trachinotus ovatus*, *Naucrates ductor* and *Polyprion americanus*.

It is difficult to accurately interpret long-term observations of coastal water thermohaline properties, considering that signals of interannual variations in seasonal temperature and salinity are strongly influenced by many agents, such as the shallowness of the water column, the freshwater inputs from rivers, the tidal amplitude and the action of wind (Orlić *et al.*, 1992). However, all changes in marine life may also suggest the changing of ocean conditions (Tonn, 1990), since marine flora and fauna integrate medium-term changes in ambient conditions (Soule & Keppel, 1988).

The status of the ribbon fish needs to be evaluated on a continuous basis, as it is becoming increasingly apparent that uncommon species can be essential indicators of environmental changes (Swabby & Potts, 1990).

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O POJAVLJANJU KOSICE *TRACHIPTERUS TRACHYPTERUS* (GMELIN, 1789) V TRŽAŠKEM ZALIVU (SEVERNI JADRAN)

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POVZETEK

V poletnih mesecih 2006 je bilo v Tržaškem zalivu zabeleženih pet odraslih osebkov kosice Trachipterus trachypterus. *Čeprav je vrsta razširjena v večjem delu tega območja, so podatki o njej redki. Avtorja opisujeta morfometrične in meristične značilnosti vrste in strukturo hrane v želodcih štirih osebkov. Napravljena je tudi primerjava z drugimi podatki o redkih vrstah v istem območju in opisan razvoj termohalinskih značilnosti v Tržaškem zalivu.*

Ključne besede: *Trachipterus trachypterus*, struktura hrane v želodcih, redka vrsta, Tržaški zaliv, Jadransko morje

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ON THE RECORD OF THE ROYAL FLAGFIN, *AULOPUS FILAMENTOSUS* (PISCES: AULOPIDAE), FROM THE ADRIATIC SEA

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ABSTRACT

The royal flagfin Aulopus filamentosus *(a single specimen, ♀: 23.7 cm total length) was caught in the southern Adriatic on 28th January 2006. This is the second confirmed record on the species, with all morphometric and meristic data, for the eastern Adriatic.*

Key words: Aulopidae, *Aulopus filamentosus*, rarity, eastern Adriatic

SEGNALAZIONE DI MERLUZZO IMPERIALE, *AULOPUS FILAMENTOSUS* (PISCES: AULOPIDAE), IN ADRIATICO

SINTESI

Il merluzzo imperiale Aulopus filamentosus *(un solo esemplare, ♀: 23,7 cm di lunghezza totale) è stato catturato nell'Adriatico meridionale il 28 gennaio 2006. Si tratta della seconda segnalazione confermata di tale specie, con tutti i dati morfometrici e meristici, per l'Adriatico orientale.*

Parole chiave: Aulopidae, *Aulopus filamentosus*, rarità, Adriatico orientale

INTRODUCTION

The royal flagfin *Aulopus filamentosus* (Bloch, 1792) is subtropical demersal species living at depths ranging from 500 to 1000 m. It occurs in the eastern Atlantic (Canary Islands south to Cape Verde and Senegal), western central Atlantic (Gulf of Mexico and Caribbean) and Mediterranean (without Adriatic and Black Seas) (Sulak, 1990). It is reported very abundant along north-western coast of Africa and off Madeira and the Canaries (Sulak, 1990). In spite of Sulak's report on the absence of royal flagfin in the Adriatic, Jardas (1985, 1996) noted its great rarity. Data on biology and ecology of the royal flagfin are very scarce.

MATERIAL AND METHODS

On 28 January 2006, a 23.7 cm total length (TL) specimen (mature ♀, still releasing eggs) of *Aulopus filamentosus* (Fig. 1) was captured by trawl at 150 m depth near Molunat Bay (southern Adriatic, Croatian coast) (Fig. 2). The specimen was identified according to Šoljan (1975). The specimen was preserved in 5% buffered formalin solution and deposited in the Ichthyological Collection of the Institute of Oceanography and Fisheries, Split; catalogue number IOR 48 – 3. This is the second confirmed record on the species for the eastern Adriatic.

RESULTS AND DISCUSSION

Body is slightly compressed, head large and robust. Eyes large and elliptical. Upper jaw reaching to eye centre, expanded posteriorly. Jaw teeth simple, short and depressible (except outer row), as also within mouth. Pseudobranch well developed. Dorsal fin high and its base is shorter than distance between dorsal and caudal fins. Pelvic fins inserted a little before origin of dorsal fin. Scales present, lateral line along midline of body. Dorsal fin set far forward and caudal fin deeply forked. Body colour is brownish-green with darker markings on flanks.

Body measurements are as follows: total length TL=23.7 cm, standard length 20.5 cm (86.6% TL), fork length 21.6 (91% TL), pre-anal length 15.0 cm (63.4% TL), pre-dorsal length 7.7 cm (32.5% TL), pre-pectoral length 5.9 cm (24.7%TL), pre-pelvic length 7.0 cm (29.5%), body depth 3.3 cm (13.9%TL), head length HL=5.4 cm (22.6%TL), eye diameter 1.4 cm (26.5%HL), pre-orbital length 1.3 cm (25.0%HL). Meristic characters are: dorsal fin rays 16, pectoral fin rays 12, pelvic fin rays 9, and anal fin rays 11. These data are the first for this species from the Adriatic Sea. All measurements are in agreement with data by Sulak (1990).

A. filamentosus is a poorly known demersal fish as far as the Adriatic Sea is concerned. Faber (1883) was the first who put this species on the list of Adriatic fish species on the basis of the record from the Boka Kotorska Bay (southern Adriatic) but without any data on the date of capture and any measurements (Pallaoro, 1988). On the basis of this list and record, other authors (Kolombatović, 1895; Ninni, 1912; Šoljan, 1975; Jardas, 1985) simply cited Faber's list of fishes. Pallaoro & Jardas (1996) registered two specimens of this species in the Ichthyological Collection of the Institute of Oceanography and Fisheries: a) single specimen (first confirmed record) caught on 3 October 1987 in Jabuka Pit (near islet Jabuka) (but without any data on measurements, type of fishing gear and donator of specimen), and b) single specimen without any other data (not confirmed record). The rarity of this species in the Adriatic could be related with its depth distribution (between 500 and 1000 m, even though it could be found in shallower waters) and availability to fishing gears. It could be

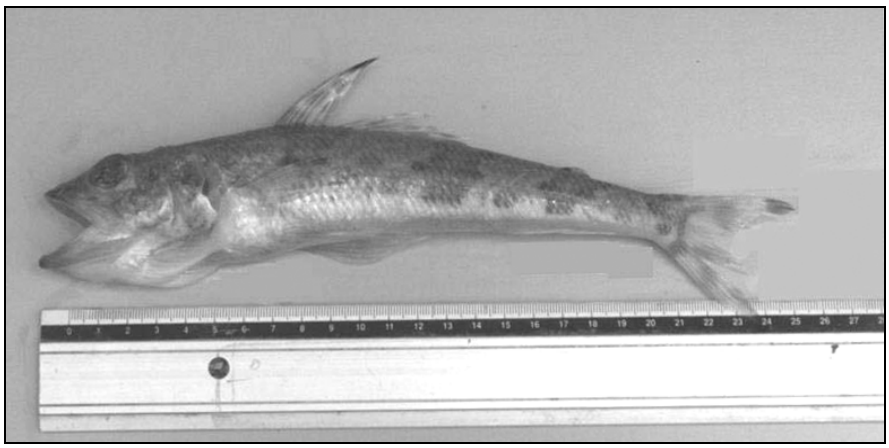

Fig. 1: Specimen of** Aulopus filamentosus**, cat. No. IOR 48 – 3, 23.7 cm TL, captured in the southern Adriatic.
Sl. 1: Primerek vrste** Aulopus filamentosus**, kat. št. IOR 48 – 3, 23,7 cm TL, ujete v južnem Jadranu.

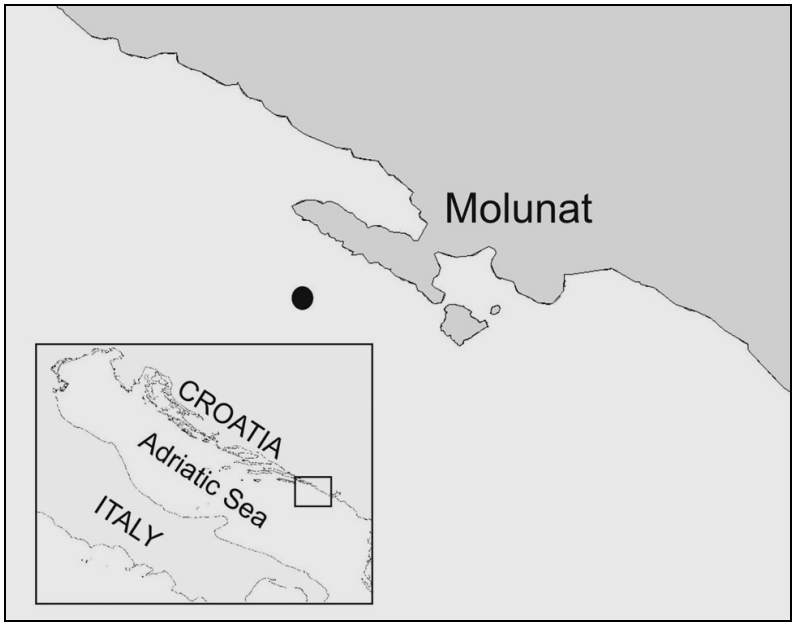

Fig. 2: Map showing the location where the specimen was captured.
Sl. 2: Zemljevid z označeno lokaliteto, na kateri je bil ujet obravnavani primerek.

pointed out that term 'rarity' is a subjective and elastic term varying with place and, moreover, it is subject to change with our increasing knowledge of the fauna. Pallaoro (1988) stated that the Adriatic ingressions caused more rare species to occur in the central Adriatic region in the1986–87 period, and among others he connected the occurrence of the royal flagfin on 3 October 1987 in Jabuka Pit with these phenomena.

O POJAVLJANJU VRSTE *AULOPUS FILAMENTOSUS* (PISCES: AULOPIDAE) V JADRANSKEM MORJU

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POVZETEK

Dne 28. januarja 2006 je bil v južnem Jadranu ujet osebek vrste Aulopus filamentosus *(en sam primerek, ♀: celotna dolžina 23,7 cm), kar je drugi potrjeni zapis o tej vrsti z vsemi morfometričnimi in merističnimi podatki za vzhodni Jadran. Redkost te vrste v Jadranu bi lahko povezali z njeno globinsko razširjenostjo in ribiško opremo, ki je na voljo ribičem, saj ta vrsta sicer živi v globinah med 500 in 1000 m. Poleg tega bi lahko poudarili, da je termin "redkost" subjektiven in raztegljiv glede na območje pojavljanja, pa tudi glede na naše vse boljše poznavanje morske favne.*

Ključne besede: Aulopidae, *Aulopus filamentosus*, redkost, vzhodni Jadran

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OBSERVATIONS ON THE BLACK-STRIPED PIPEFISH *SYNGNATHUS ABASTER,* RISSO 1826 (SYNGNATHIDAE) FROM TUNISIAN WATERS (CENTRAL MEDITERRANEAN)

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ABSTRACT

Morphometric measurements, counts and a short description of specimens of the black-striped pipefish Syngnathus abaster *Risso, 1810, caught in Tunisian waters, are presented. The occurrence of the species in the area is commented and discussed.*

Key words: Osteichthyes, Syngnathidae, *Syngnathus abaster*, morphometry, Tunisia, Mediterranean Sea

OSSERVAZIONI DI PESCE AGO CODAGROSSA *SYNGNATHUS ABASTER,* RISSO 1826 (SYNGNATHIDAE) IN ACQUE TUNISINE (MEDITERRANEO CENTRALE)

SINTESI

L'articolo presenta i dati morfometrici ed una breve descrizione degli esemplari di pesce ago codagrossa Syngnathus abaster *Risso, 1810, catturati in acque tunisine. La presenza della specie nell'area in questione viene commentata e discussa.*

Parole chiave: Osteichthyes, Syngnathidae, *Syngnathus abaster*, morfometria, Tunisia, mare Mediterraneo

INTRODUCTION

The black-striped pipefish *Syngnathus abaster* (Risso, 1810) was included by Bradaï (2000) and Bradaï *et al.* (2004) among the five syngnathid species reported in the Tunisian waters. *S. abaster* was first sighted by Seurat (1934) in estuarine waters running into the Gulf of Gabès (southern Tunisia) and eventually described by D'Ancona (1934) from specimens deposited in the Ichthyological Collection of the Station Océanographique of Salammbô, 15 km north of Tunis, at present known as Institut National des Sciences et Technologies de la Mer. Chaouachi & Ben Hassine (1998) mentioned *S. abaster* only in a check-list of fishes from the Ichkeul Lagoon (northern Tunisia). Unfortunately, the *S. abaster* specimens formerly preserved in the Ichthyological Collection of the Institut National des Sciences et Technologies de la Mer of Salammbô (see D'Ancona, 1934) do not exist at the Institute any more. Although previous reports on *S. abaster* are not doubtful, no specimens had been available until recently in Tunisia for confirmation. Investigations carried out during five years on syngnathids from Tunisian waters enabled us to find new specimens of *S. abaster*, to confirm the occurrence of the species in the area, and to provide additional data in order to expand the knowledge of the species.

MATERIAL AND METHODS

The syngnathid species constituted the focus of investigations conducted between 2001 and 2006 (Ben Amor *et al.*, *in press*), at least three times per week, in Tunisian waters (Fig. 1). One hundred and three *Syng-*

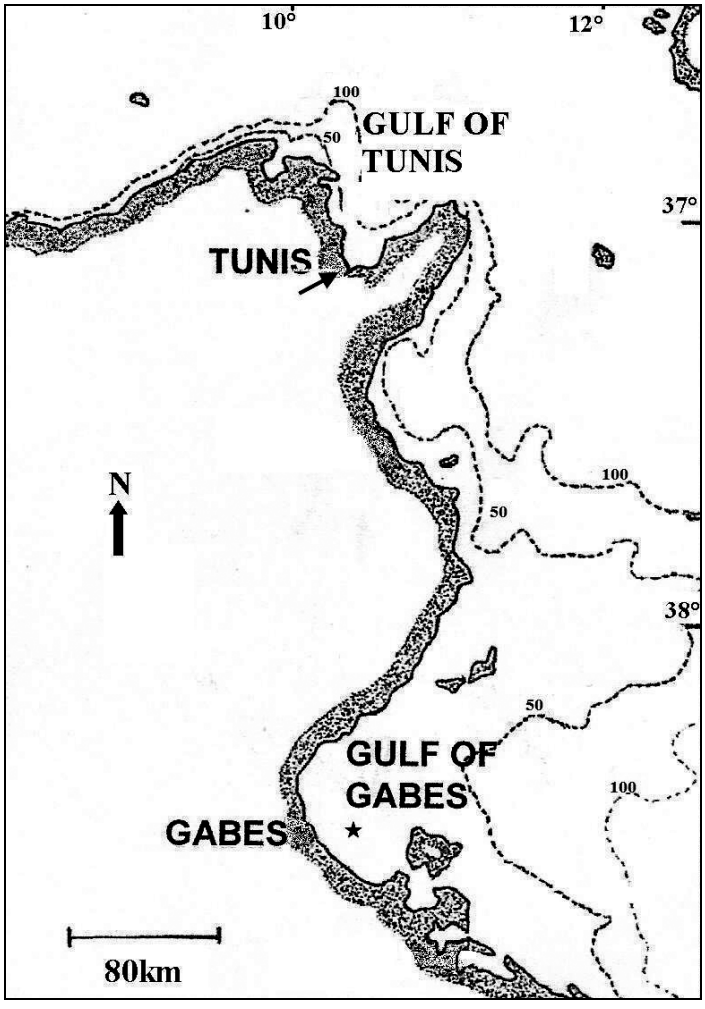

Fig. 1: Map of Tunisia showing the Tunis Southern Lagoon site (black arrow), and the fishing site of the specimen caught in the Gulf of Gabès (black star).
Sl. 1: Zemljevid Tunizije z lokaliteto v Tuniški južni laguni (črna puščica) in lokaliteto v Gabeškem zalivu (črna zvezdica), kjer so bili ujeti osebki malega šila.

nathus abaster individuals were collected from the Tunis Southern Lagoon using spoons, drags and SCUBA dive, and a single one in the Gulf of Gabès, by trawl, on 24 April 2005, at a depth between 25–30 m above the seagrass meadow overgrown particularly by *Cymodocea* sp. and *Caulerpa* sp.

Of the 104 collected Tunisian specimens, only 40 were subjected to morphometric measurements to the nearest millimetre and meristic counts plotted in Figure 2. The latter specimens were preserved in 5% buffered formalin and deposited in the Ichthyological Collection of the Faculté des Sciences of Tunis, catalogue number FST-SYN-Abaster-01 to FST-SYN-Abaster-40. Measurements and counts carried out on the Tunisian specimens are compared with those carried out on 27 black-striped pipefishes preserved in the Ichthyological Collection of the Muséum d'Histoire Naturelle of Paris (MNHN). Of the 27 MNHN specimens, 5 were from Algeria, 2 from Italy, 4 from France, 2 from the Adriatic Sea, 4 from the Aegean Sea and 3 from Romania.

Test for significance ($p < 0.05$) were performed using Student's t-test, Snedecor's F-test. Linear regression was performed following log transformation of data. Correlations were assessed by least-squares regression. Curves were compared by ANCOVA.

RESULTS

Description of the Tunisian specimens

Body elongate, rather rounded (Fig. 3), head-length 2.32–4.81 in pre-anal length, 5.35–12.80 in total-length; snout slightly rounded without knobs, but with an inconspicuous keel on upper surface, snout-length 1.50–5.67 in head-length; eye rounded and minute, 3.71–8.86 in head-length; pre-orbitary length 1.22–2.75 in head-length; post-orbitary length 1.59–3.31 in head-length; pre-dorsal length 2.20–2.96 in total-length; dorsal base 7.02–14.06 in total length, dorsal length 0.76–0.98 in dorsal base; pre-anal length 2.05–3.02 in total-length; dorsal fin slender with 16–35 soft rays on 5–7 rings; pectoral with 10–16 soft rays; anal with 3–4 soft rays; caudal with 9–13 soft rays; anus located under the beginning of the dorsal fin; 14–18 rings before anus, 24–37 trunk rings, 39–53 total rings.

Colour greenish to brownish or reddish, body with white lines and spots, snout rather brownish, with a blackish spot before eyes, black spots arranged in a line under the dorsal base. Belly whitish or beige.

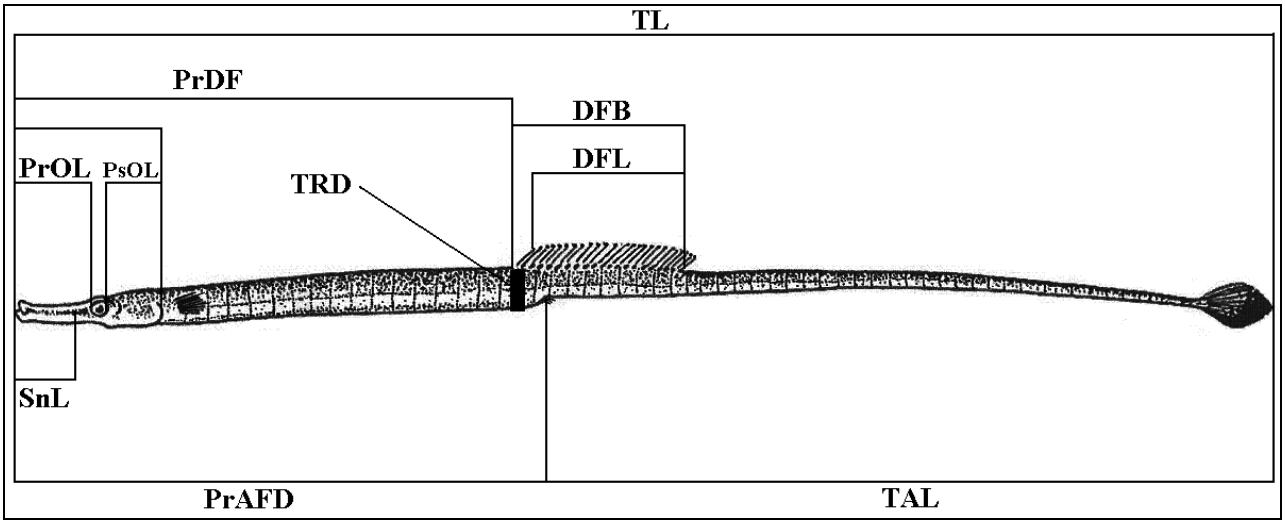

Fig. 2: Measurements carried out on** S. abaster **following Dawson (1982) for syngnathids. Specimen redrawn from Tortonese (1970). Legend: DFB – dorsal fin base; DFL – dorsal fin length; ED – eye diameter; HL – head length; Pr AFD – pre-anal fin distance; Pr DF – pre-dorsal fin distance; Pr OL – pre-orbitary length; Ps OL – post-orbitary length; SnL – snout length; TAL – tail length; TL – total length; TRD – trunk depth.
Sl. 2: Meritve, opravljene na malem šilu v skladu z Dawsonovo metodologijo (1982) za družino Syngnathidae. Primerki so narisani na novo po Tortoneseju (1970). Legenda: DFB – baza hrbtne plavuti; DFL – dolžina hrbtne plavuti; ED – premer očesa; HL – dolžina glave; Pr AFD – razdalja predanalne plavuti; Pr DF – razdalja predhrbtne plavuti; Pr OL – preorbitalna razdalja; Ps OL – postorbitalna razdalja; SnL – dolžina gobca; TAL – dolžina repa; TL – celotna dolžina; TRD – višina trupa.

There were positive relationships total length *versus* total mass (TM) and total length *versus* eviscerated mass (EVM); these relations did not significantly differ between them. The relationships were: Log TM = 2.622 Log TL – 12.59; r = 0.92; n = 104 and Log EVM = 2.737 Log TL –13.499; r = 0.89; n = 104.

Comparisons between Tunisian specimens and specimens from MNHN

Specimens from MNHN were from six different origins, but as they were not statistically supported by sufficient data to be compared with the Tunisian specimens, they were included in the same sample. Some relations were compared between Tunisian sample and MNHN sample, such as HL/PrAFD; HL/TL; SnL/HL; PrAFD/TL; PrDF/TL; PrOL/HL; PsOL/HL; DFL/DFB; DFB/TL; ED/HL, as well as all meristic counts (see Fig. 2).

HL/TL, PrAFD/TL, PrDF/TL, DFB/TL, dorsal fin soft rays and pectoral fin soft rays significantly differed between Tunisian and MNHN samples.

DISCUSSION

Description, morphometric measurements and meristic counts are in agreement with Tortonese (1970), Bauchot & Pras (1980) and Dawson (1982, 1986).

Although there were significant differences between specimens from Tunisian waters and those from MNHN of Paris, it is difficult to state that they belong to different populations: the second sample comprised specimens of six different origins. However, Cakic *et al.* (2002) showed significant differences in similar morphometric characters between specimens from the Black Sea and Ukrainian freshwaters.

Dawson (1982, 1986) noted that the straight-nosed pipefish inhabits shallow coastal waters, coasts and estuaries, usually at 4–20 m. The Tunis Southern Lagoon was recently subjected to environmental restoration that allowed colonization of fish species previously unknown in the area (Ben Souissi *et al.*, 2004, 2005; Mejri *et al.*, 2004). *S. abaster* must be included among them. In the area, the species found sufficient resources to subsist and probably to develop and reproduce in brackish areas, as shown by positive relationships total length *versus* total mass and total length *versus* eviscerated mass. Similar patterns were described by Tomasini *et al.* (1991) for specimens collected in the Lagoon of Mauguio (southern France), Koutrakis & Tsikiras (2003) from northern Aegean estuarine waters, and Verdiell-Cubedo *et al.* (2006) from Mar Menor coastal lagoon (western Mediterranean Sea).

S. abaster was previously reported throughout the southern coast of the Mediterranean, such as off Algeria (Dieuzeide *et al.*, 1954), Libya (Al Hassan & El Silini, 1999), Egypt (El Sayed, 1994) and Israel (Golani, 2005). Our investigations have shown that the species was caught predominantly in protected areas and only rarely outside them, as shown by a single specimen caught in the Gulf of Gabès (see above). This suggests that the species was probably sensitive to both interspecific and anthropic pressures. However, the species was generally discarded at sea by fishermen and probably escaped observations; this could also explain its scarcity in marine waters. Our observations allow us to confirm the occurrence of *S. abaster*, not only as a new teleost species found in the Tunis Southern Lagoon (see Ben Souissi *et al.*, 2005), but also in Tunisian waters, in agreement with D'Ancona (1934), Seurat (1934) and Chaouachi & Ben Hassine (1998).

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The authors wish to thank two anonymous referees for their thorough revision of the ms, which allowed us its considerable improvement.

Fig. 3: *S. abaster* ***(FST-SYN-Abaster 06) collected in the Tunis Southern Lagoon.***
Sl. 3: *S. abaster* ***(FST-SYN-Abaster 06), ujet v Tuniški južni laguni.***

O POJAVLJANJU MALEGA ŠILA *SYNGNATHUS ABASTER*, RISSO 1826 (SYNGNATHIDAE) V TUNIZIJSKIH VODAH (SREDNJE SREDOZEMLJE)

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POVZETEK

Avtorji predstavljajo morfometrične meritve in kratek oris osebkov malega šila Syngnathus abaster *Risso, 1810, ujetih v tunizijskih vodah. Hkrati razpravljajo o pojavljanju vrste v tem območju.*

Ključne besede: Osteichthyes, Syngnathidae, *Syngnathus abaster*, morfometrija, Tunizija, Sredozemsko morje

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ANTIOXIDANTS IN VIRGIN OLIVE OILS PRODUCED FROM TWO OLIVE CULTIVARS OF SLOVENE ISTRIA

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ABSTRACT

The content of biophenols and tocopherols in virgin olive oils is an important factor when evaluating their quality. In the present work, the differences in biophenols and tocopherols content of two major olive tree (Olea europaea *L.) cultivars in Slovene Istria – cv. 'Istrska belica' and 'Leccino' – based on 1997/98 and 1998/99 crops were examined. It was established that cv. 'Istrska belica' had higher total biophenols content than the tocopherols content compared to the cv. 'Leccino', which had higher tocopherols content. Furthermore, the differences in the extraction processes on the biophenols and tocopherols content in virgin olive oils crops 1999/2000 and 2000/2001 were examined, showing the dual phase decanter (DP) process gives better results. And – finally – the inadequate storage conditions (light and room temperature) effect was measured, confirming that the direct light speeds up the biophenols and tocopherols decomposition.*

Key words: virgin olive oil, biophenols, tocopherols, extraction process, HPLC, Slovene Istria

ANTIOSSIDANTI NEGLI OLII VERGINE D'OLIVA DELL'ISTRIA SLOVENA DI DUE CULTIVAR D'OLIVO

SINTESI

La quantità di biofenoli e tocoferoli contenuta nell'olio d'oliva è importante nella stima della sua qualità. L'articolo riporta il confronto tra le quantità di biofenoli e tocoferoli contenute in due cultivar dell'olivo (Olea europaea *L.), che crescono nell'Istria slovena – 'Bianchera Istriana' e 'Leccino' – negli anni 1997/98 e 1998/99. Gli autori hanno confermato che la quantità di biofenoli negli olii di Bianchera Istriana è più alta di quella contenuta negli olii di Leccino. Questi ultimi hanno però una quantità più alta di tocoferoli. Gli autori hanno inoltre confrontato l'influsso del processo di estrazione sul contenuto in biofenoli e tocoferoli e hanno dimostrato che il processo bifasico porta ad olii con una maggiore quantità di biofenoli e tocoferoli. Viene inoltre evidenziato l'effetto di un errato deposito (calore e luce) sulla quantità di biofenoli e tocoferoli negli olii e viene dimostrato che la luce diretta porta ad un aumento del decadimento di biofenoli e tocoferoli negli olii.*

Parole chiave: olio vergine d'oliva, biofenoli, tocoferoli, HPLC, produzione, Istria slovena

INTRODUCTION

Slovene Istra is the coastal part of Slovenia in the upper part of the Adriatic in the Mediterranean basin, world known for its diet based on virgin olive oils (VOO). Virgin olive oils contain many compounds of non triacylglycerolic origin – among them are dietary antioxidants biophenols (BP) and tocopherols (TOC) (Baldioli *et al.*, 1996; Tasioula-Margari & Okogeri, 2001). Virgin olive oil tocopherols are mostly (> 95%) composed of alpha-tocopherol isomer (Boskou, 1996). The content of biophenols (Uccella, 2001a, 2001b, 2001c) and tocopherols (Butinar *et al.*, 1999b) in virgin olive oils plays an important role when evaluating their overall quality. Biophenols are known to improve resistance to autooxidation, and they give the oil its characteristic fresh, fruity, piquant (sometimes fiercely fruity) flavour (Angerosa *et al.*, 2000). These compounds have a role in disease prevention (Owen *et al.*, 2000a) and prolong the shelf life of the virgin oil itself by preventing auto oxidative radical reactions (Blekas *et al.*, 1995; Dietary Reference Intakes, 2000; Caponio *et al.*, 2001).

The prevailing virgin olive oil biophenols are phenolic secoiridoide glucosides (SG). Both olive biophenols and tocopherols are secondary metabolites deriving from AcCoA (mevalonic acid) and phosphoenolpyruvate (shikimic acid) pathways (Ryan & Robards, 1998).

Olive biophenols from secoiridoide glucosides pathway are mainly oleuropein (structure 1 from Fig. 1), demethyloleuropein, ligstroside (structure 2 from Fig. 1), oleoside and their decomposition – hydrolytic/enzymatic products that during the processing of olives enter the lipophylic phase and enrich the virgin olive oils: among them are oleuropein aglycon (O-Agl) in two different tautomeric forms: hidroxy (structure 1b from Fig. 1) and aldehydic (structure 1a from Fig. 1), ligstroside aglycon (L-Agl), again in two different forms: hidroxy (structure 2b from Fig. 1) and aldehydic (structure 2a from Fig. 1), dialdehydic open form of decarboxymethyl oleuropein aglycon (DMO-dA) represented with the structure 1d from Fig. 1, dialdehydic open form of decarboxymethyl ligstroside aglycon (DML-dA) with the structure 2d from Fig. 1, tyrosol (Tyr) – structure 4 from Fig. 2 and hydroxytyrosol (Tyr-OH) – structure 3 from Fig. 2 (Cortesi *et al.*, 2002; Rovellini & Cortesi, 2002). The virgin olive oils' biophenolic segment is rich in lignans and two flavonoids: apigenin and luteolin (Cortesi *et al.*, 2002; Owen *et al.*, 2000b).

The content of biophenols and tocopherols in freshly pressed virgin olive oils depends on many factors – among them are the cultivar, cropping year, fruit ripeness and overall fruit condition (Gimeno *et al.*, 2002; Salvador *et al.*, 2003), climatic conditions (Patumi *et al.*, 2002; Manach *et al.*, 2004; Paz Aguilera *et al.*, 2005) and type and quality of the extraction process (Gutiérez

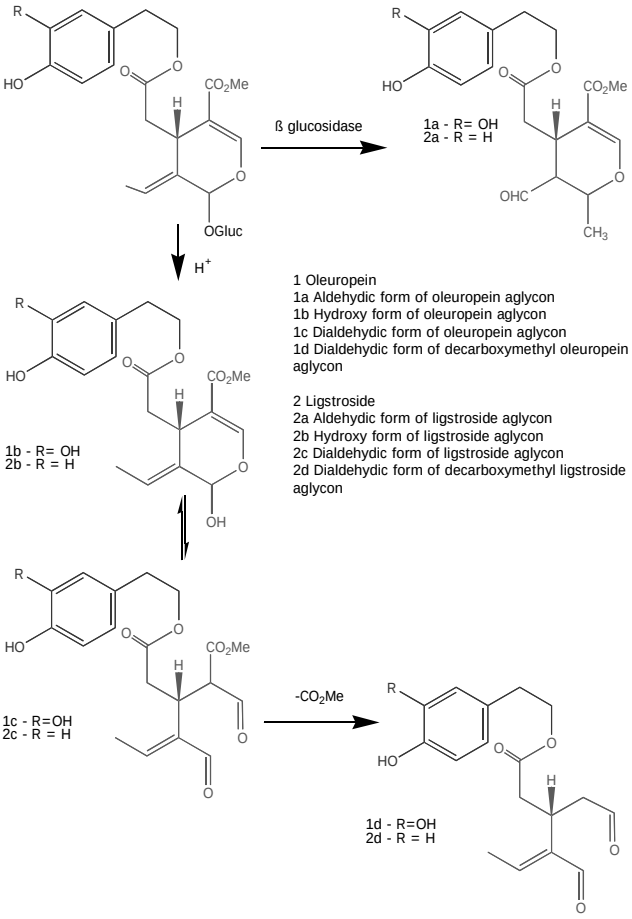


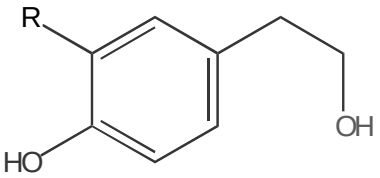
Fig. 1: Formation mechanisms of oleuropein and ligstroside biophenolic aglycons (Rovellini & Cortesi, 2002).

Sl. 1: Tvorbeni mehanizmi oleuropeinskih in ligstrozidnih biofenolnih aglikonov (Rovellini & Cortesi, 2002).

et al., 1999; Caponio & Catalano, 2001; Ranalli *et al.*, 2001). During storage, the content of simple biophenols Tyr and Tyr-OH depends on hydrolytic and enzymatic processes transforming complex secoiridoid glucosides in less complex ones (decomposition path as illustrated in Fig. 1) and on the oxidation of simple ortho biophenols – hydroxytyrosol. Tyrosol has negligible antioxidative activity, thus its content remains practically unchanged or even slightly increases due to ligstroside complex biophenols decomposing.

The aim of the present work was to examine the differences in biophenols and tocopherols content of the two major olive tree (*Olea europaea* L.) cultivars in Slovene Istra – cv. 'Istrska belica' (IB) and 'Leccino' (L) – based on 1997/98 and 1998/99 crops and to compare them to the values found in previous published work (Butinar *et al.*, 1999a, 1999b). Furthermore, we examined the effect of different extraction processes on bio-

phenols and tocopherols content in virgin olive oils and the effect of inadequate storage conditions (light and room temperature) on total biophenols and tocopherols content and on content of HPLC determined secoiridoide originated biophenols as well.



3 - R=OH
4 - R=H

Fig. 2: Structures of tyrosol (4) and hydroxytyrosol (3).
Sl. 2: Strukturi tirosola (4) in hidroksitirosola (3).

MATERIALS AND METHODS

Materials

Cultivar influences. The research was performed on 21 virgin olive oil samples from the 1997/98 crop, analyzed in September 1998, and on 26 virgin olive oil samples from the 1998/99 crop, analyzed in October 1999. All virgin olive oil samples were stored in the dark, packed in dark-coloured air tight bottles at 15 °C. All virgin olive oil samples were pressed from the cv. 'Istrska belica' and 'Leccino' olives, or were mixtures of both cultivars that underwent the extraction process as a mixture.

Extraction process evaluation. The first part of evaluation was performed on oils from cv. 'Istrska belica' and 'Leccino' crop 1999/00, processed in two different olive-mills, first one using the percolation/centrifugation process (PC) and the second the centrifugal integral decanter (dual phase decanter, DP).

The second part of evaluation was performed on oils from cv. 'Istrska belica' and 'Leccino' crop 2000/01, processed in two commercially run olive-mills, both using the same extraction principle: centrifugal integral decanter, but being run under slightly different operation conditions due to each owner's strategies (DP-1 & DP-2).

Inadequate storage effects. The biophenols determinations were made on initially 21 virgin olive oil samples crop 1997/98, analyzed for the first time in September 1998. 11 out of those 21 samples were analyzed for the second time in May 1999, after being stored at 18 °C in dark-coloured bottles, and 6 out of these 11 samples were analyzed for the third time in May 2001, after being kept in transparent bottles at 18 °C.

The tocopherol determination on 4 virgin olive oil samples crop 1998/99 was performed for the first time in February 1999. The samples were stored in dark-coloured bottles in dark and cold place till June 1999 when the aliquot of the samples was put into transparent bottles and left on the laboratory shelf in full light until analyzed in May 2001.

Reference compounds. Tocopherol standards were obtained from Merck (Darmstadt, Germany). The calibration standard concentrations were spectrophotometrically checked according to A.O.C.S. method Ce 8–89 (AOCS, 1990) and (Balz *et al.*, 1996). Tyrosol was purchased from Fluka (Buchs, Switzerland). Hydroxytyrosol was prepared in our laboratory according to the publication of Baraldi (Baraldi *et al.*, 1983) and its purity checked with the aid of HPLC.

Methods

Extraction of biophenolic compounds and determination of total biophenols. The extraction and determination were performed according to Gutfinger's publication (Gutfinger, 1981).

HPLC analysis of biophenols. 100 µl of the biophenols extract (prepared as in 3.3.1) were put in the vial of the auto sampler of an Agilent 1050 quaternary pump HPLC system, equipped with an UV/VIS detector operating at 280E-9 m and Supelco 250 mm × 4.6 mm ODS column. 25 – 75 µl were injected into the system, the flow rate set to 1.0 ml/min, and the mobile phase used was a water/acetic acid and methanol gradient, which allowed the separation of the simple (Tyr and Tyr-OH) biophenols from the complex ones. The quantization of tyrosol and hydroxytyrosol was carried out by the external standard method. The response factor of tyrosol was used to quantitate the dialdehydic open form of decarboxymethyloleuropein aglycon and other biophenols.

Extraction of tocopherols. 500 mg of virgin olive oil sample were weighed in a 10 ml vial and 5.0 ml of methanol were added. The vial was capped and sonicated for 10 minutes. The methanol extract was refrigerated to allow the oil droplets to settle, filtered and transferred to the auto sampler vial (The New Expanded Supelco Reporter, 1993).

HPLC analysis of tocopherols. We used an Agilent system, equipped with a quaternary pump, auto injector and UV/VIS detector. Separation was achieved on a Supelco ODS 5µm, 4.6 x 250 mm column. The eluate absorbance was monitored at 290E-9 m. The mobile phase consisted of 2 vol. % MeOH in water. The flow during the analysis was 2 ml/min and the time necessary to separate all the peaks 10 minutes. The injection volume ranged from 50 – 75 µl of the methanol extract. The quantization of tocopherols (beta and gamma isomer co eluted) was carried out by the external standard method. For the alpha-tocopherol a 5 point calibration curve was constructed, which showed the R^2 = 0.9998 linearity in the range from 1 to 17 mg of the isomer/100 g of the oil.

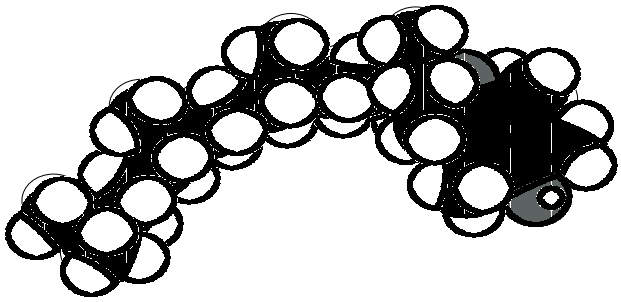


Fig. 3: Structure of alpha-tocopherol (white circles represent hydrogen atoms, black circles carbon and dotted circles oxygen atoms).
Sl. 3: Struktura alfa-tokoferola (beli krogci so vodikovi atomi, črni ogljikovi in šrafirani kisikovi).

RESULTS

Cultivar differences

Figures 4 and 5 show the average total biophenols content in virgin olive oils from the 1997/98 and 1998/99 crops and their comparison with tocopherols (Figs. 6 and 7). There are obvious differences between 'Leccino' and 'Istrska belica'. Virgin olive oils extracted from cultivar 'Leccino' have higher share in total tocopherols content compared to 'Istrska belica' oils, while in total biophenols content the ratio is turned around – 'Istrska belica' oils lead in total biophenols content.

Extraction process evaluation

Table 1 summarizes the cultivar, type of processing, total biophenols content, total tocopherols content, hydroxytyrosol, tyrosol, dialdehydic open form of decarboxymethyl oleuropein aglycon (DMO-dA) and total HPLC biophenols content for samples examined. The biophenols and tocopherols content are higher in both IB and L cultivars when using the dual phase decanter extraction process.

Tab. 1: Extraction processes data for virgin olive oil samples crop 1999/00: PC – percolation/centrifugation process; DP – centrifugal integral decanter (dual phase decanter).
Tab. 1: Podatki iz ekstrakcijskega procesa za vzorce deviškega oljčnega olja letnika 1999/00: PC – perkolacijsko/centrifugalni proces; DP – centrifugalno integralni dekanter (dvofazni dekanter).

Content	Cultivar			
	Istrska belica		Leccino	
	PC	DP	PC	DP
Total BP (mg/kg)	127	153	59	75
Total TOC (mg/100 g)	3.3	4.1	6.2	9.1
TyrOH (mg/kg)	5.9	3.7	0.4	1
Tyr (mg/kg)	12.9	7.0	4.9	6.1
DMO-dA (mg/kg)	4.9	14.9	0	0
Total HPLC BP (mg/kg)	164	213	80	90

Table 2 shows the cultivar, type of processing, total BP content, total TOC content, hydroxytyrosol, tyrosol, dialdehydic open form of decarboxymethyl oleuropein aglycon (DMO-dA) and total HPLC biophenols content for samples examined. Note the elevate total tocopherol amount in the extraction process DP-2 (probably due to added vegetation water during extraction process).

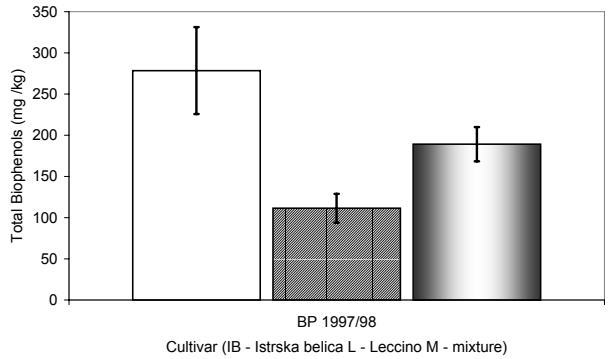


Fig. 4: Average total biophenol content for the oils from cultivars 'Istrska belica', 'Leccino' and mixtures of both produced from the 1997/98 crop olives (the error bars show the standard deviation).
Sl. 4: Povprečni skupni biofenoli v oljih sort 'Istrska belica', 'Leccino' in mešanic obeh iz oljk letnika 1997/98 (standardni odklon je označen z daljicami).

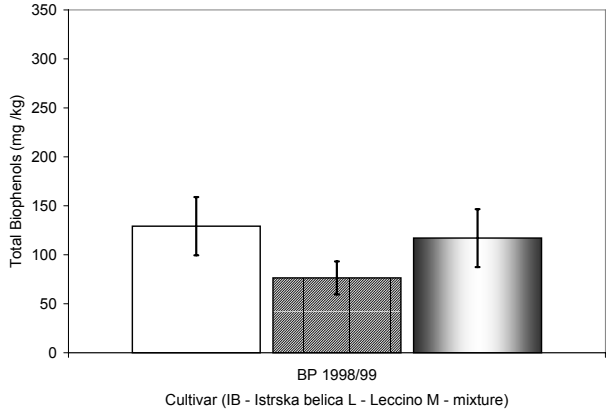


Fig. 5: Average total biophenol content for the oils from cultivars 'Istrska belica', 'Leccino' and mixtures of both produced from the 1998/99 crop olives (the error bars show the standard deviation).
Sl. 5: Povprečni skupni biofenoli v oljih sort 'Istrska belica', 'Leccino' in mešanic obeh sort iz oljk letnika 1998/99 (standardni odklon je označen z daljicami).

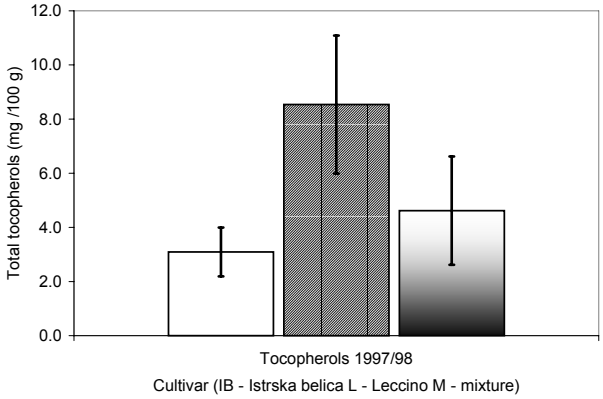


Fig. 6: Average total tocopherol content in the virgin olive oils from 'Istrska belica', Leccino' and mixtures of both cultivars from the 1997/98 crop (the error bars show the standard deviation).
Sl. 6: Povprečna skupna vsebnost tokoferolov v deviških oljčnih oljih sort 'Istrska belica', Leccino' in mešanic obeh sort letnika 1997/98 (standardni odklon je označen z daljicami).

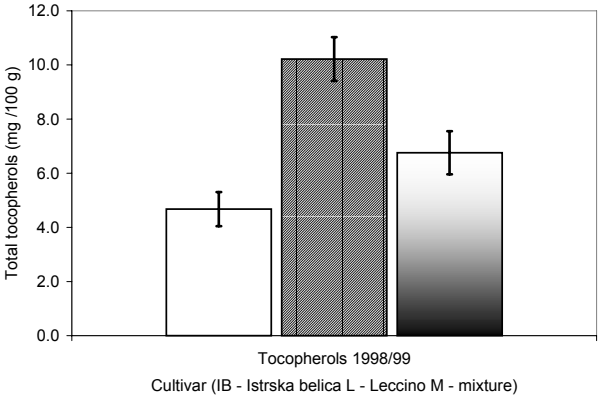


Fig. 7: Average total tocopherol content in the virgin olive oils from 'Istrska belica', Leccino' and mixtures of both cultivars from the 1998/99 crop (the error bars show the standard deviation).
Sl. 7: Povprečna skupna vsebnost tokoferolov v deviških oljčnih oljih sort 'Istrska belica', Leccino' in mešanic obeh sort letnika 1997/98 (kazalci so standardni odmik).

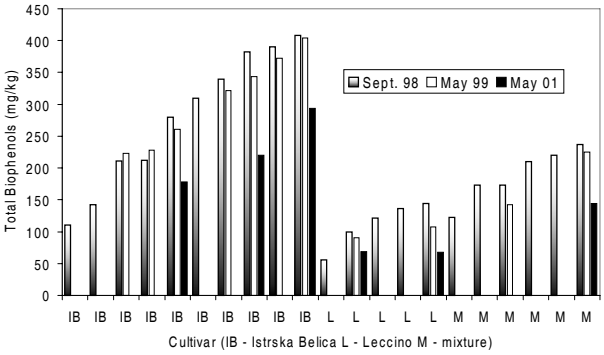


Fig. 8: Changes in the total biophenol content in selected virgin olive oils from the 1997/98 crop during 18 months of storage.
Sl. 8: Spremembe vsebnosti skupnih biofenolov v izbranih deviških oljčnih oljih letnika 1997/98 med 18-mesečnim hranjenjem.

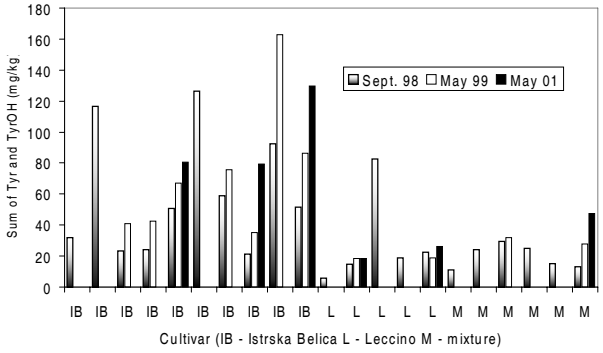


Fig. 9: Changes of the sum of Hydroxytyrosol and Tyrosol in selected virgin olive oils from the 1997/98 crop during 18 months of storage.
Sl. 9: Spremembe vsote hidroksitirosola in tirosola v izbranih deviških oljčnih oljih letnika 1997/98 med 18-mesečnim hranjenjem.

Tab. 2: Extraction processes data for virgin olive oil samples crop 2000/01: DP – centrifugal integral decanter (dual phase decanter).
Tab. 2: Podatki iz ekstrakcijskega procesa za vzorce deviškega oljčnega olja letnik 2000/01: DP – centrifugalno integralni dekanter (dvofazni dekanter).

Content	Cultivar			
	Istrska belica		Leccino	
	DP-1	DP-2	DP-1	DP-2
Total BP (mg/kg)	218	144	134	84
Total TOC (mg/100 g)	6.1	10	7.5	10,4
TyrOH (mg/kg)	5.4	11.3	2.9	3.4
Tyr (mg/kg)	4.3	8.6	9.2	6.5
DMO-dA (mg/kg)	87.6	41	37.9	13.8
Total HPLC BP (mg/kg)	279	193	233	125

Inadequate storage effects

Biophenols. We monitored changes in virgin olive oil samples crop 1997/98 in 3 time determinations. Figures 8, 9 and 10 show various biophenols relations in the ageing processes.

The 2 arrows in Figue 10 show the 'Leccino' samples running out of hydroxytyrosol, meaning that they lost their antiooxidative power from the biophenols species. The sum of tyrosol and hydroxytyrosol concentration in Figure 9 is rising, which demonstrates that the complex phenols are transformed into 'simple' ones and that Tyr is not being consumed, thus showing not being antioxidative potent.

Tocopherols. Figure 11 shows the influence of light on total tocopherols decomposition in virgin olive oil samples of 'Istrska belica' and 'Leccino' from crop 1998/99. The transparent bottle facilitates the passage of light into oil thus speeding the decomposition.

DISCUSSION AND CONCLUSIONS

Comparison of total biophenols content in the virgin olive oil samples from the olives harvested in the 1997/98 and 1998/99 crop years confirmed that several factors can influence the content of biophenols, such as: climatic conditions, harvesting and extraction process. Taking in regard the fact that olive orchards, cultivars, extraction facilities and harvesting time remained practically unchanged in the two crop seasons, we can speculate and conclude the climate has a major impact on the biophenols content, and even more so if we consider the fact biophenols are polar substances that can be rather easily leached out from the fruits or in the extraction process phase if their starting amount is relatively low. Secondly – the relative amount of total biophenols in the virgin oils processed from the cultivar 'Istrska belica' compared to the total biophenols content in the virgin oils processed from the cultivar 'Leccino' stays always higher, no matter what the actual absolute value might be (Figs. 4 and 5). When the total tocopherols content is considered, the virgin olive oils processed from cultivar 'Leccino' lead when compared to the oils processed from the cultivar 'Istrska belica', thus confirming our previous findings (Butinar *et al.*, 1999b).

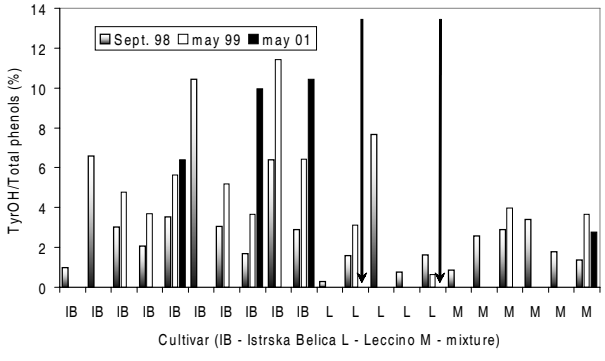


Fig. 10: Changes of the ratio Tyr-OH/total HPLC biophenols in virgin olive oils from the 1997/98 crop during 18 months of storage.
Sl. 10: Spremembe razmerja Hidroksitirosol/skupni HPLC biofenoli deviških oljčnih oljih letnika 1997/98 med 18-mesečnim hranjenjem.

It was shown how different centrifugation extraction processes can influence the total and HPLC biophenols content: percolation/centrifugation process vs. centrifugal/integral decanter process (Tab. 1) and how various ways of performing the same centrifugal/internal decanter (dual phase decanter) – DP-1 and DP-2 process can influence it as well (Tab. 2). It can be concluded that the process DP-2 was run with more water added compared to process DP-1 thus leaching the polar biophenols out of the oil. Consequently, the oil from process DP-1 is richer in total biophenols, total HPLC biophenols and complex biophenols (DMO-dA) meaning the amount of water added did not hydrolytically decompose the complex biophenols to simple ones (Tyr & TyrOH). The data for TyrOH in Table 2 demonstrate this: 5.4 *vs.* 11.3 mg/kg in IB oils and 2.9 *vs.* 3.4 mg/kg in L oils.

Figures 8, 9 and 10 clearly show how storage (inadequate storage conditions) influence the degradation process of complex biophenols towards the simple ones resulting in elevated degrees of hydroxytyrosol and tyrosol in the first stages then gradually changing to diminution of hydroxytyrosol degree (being antioxidatively active) not influencing the decay of tyrosol (not being antioxidatively active). The total biophenols content in some samples after 9 months of storage slightly increased (samples 3 and 4 for IB in Fig. 8). This can be explained either by considering the measurement uncertainty for the total BP determination (the differences being somewhat small) or by the fact the simple and complex biophenols have slightly different extinction coefficients when determining the absorbency in the total biophenols content determination using FC reagent. In the future work it would be more proper to report molar concentrations and not the mass ones.

The light and room temperature can considerably speed up the tocopherols decomposition; the light not absorbed in the dark-coloured bottles speeding the decomposition process even more (*e. g.* Fig. 11).

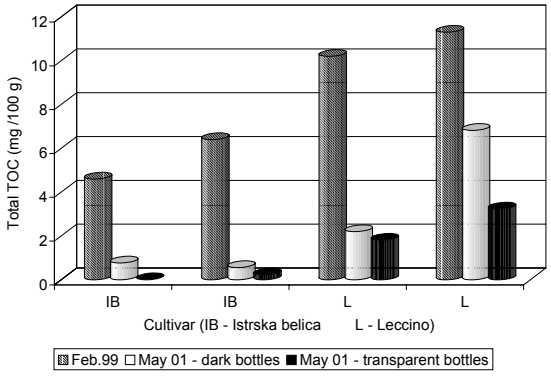


Fig. 11: Influence of light on the total tocopherol content in virgin olive oils from cv. 'Istrska belica' and cv. 'Leccino'.
Sl. 11: Vpliv svetlobe na vsebnost skupnih tokoferolov deviških oljčnih olj sort 'Istrska belica' in 'Leccino'.

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POVZETEK

Slovenska Istra je del Mediterana, ki je svetovno znan po svoji specifični prehrani. Ena njenih najpomembnejših sestavin je deviško oljčno olje. Deviško oljčno olje vsebuje tudi sestavine netriacilglicerolnega izvora – med njimi za kakovost zelo pomembne antioksidante biofenole in tokoferole. Poznano je, da biofenoli povečujejo odpornost proti antioksidaciji in da olju dajejo značilen okus in vonj. Biofenoli in tokoferoli deviško oljčno olje ščitijo pred kvarjenjem, saj preprečujejo/dušijo reakcije avtooksidacije. Biofenoli deviških oljčnih olj so pretežno sekoiridoidno glukozidnega izvora. So oleuropein, ligstrozid, oleozid in predvsem v olju njihovi razpadni produkti: oleuropein aglikon, ligstrozid aglikon, odprta dialdehidna oblika dekarboksimetil oleuropein aglikona, odprta dialdehidna oblika dekarboksimetil ligstrozid aglikona, hidroksitirosol in tirosol. Na vsebnost biofenolov in tokoferolov v deviškem oljčnem olju vpliva veliko dejavnikov. Med hrambo se vsebnosti hidroksitirosola in tirosola spreminjata. Antioksidativna aktivnost tirosola je zanemarljiva, zato se njegova vsebnost v olju praktično ne spreminja ali pa se le rahlo povečuje. V pričujočem prispevku smo primerjali vsebnost biofenolov in tokoferolov v dveh kultivarjih oljke (Olea europaea *L.), ki uspevata v Slovenski Istri – 'Istrska belica' in 'Leccino' – in sicer v dveh zaporednih letnikih 1997/98 in 1998/99 s podatki iz naših prejšnjih objav in jih potrdili. Potrdili smo, da je vsebnost biofenolov v oljih iz sorte 'Istrska belica' višja od vsebnosti biofenolov v oljih iz sorte 'Leccino', ki pa imajo višjo vsebnost tokoferolov v primerjavi z olji iz sorte 'Istrska belica'. Primerjali smo tudi vpliv ekstrakcijskega procesa na vsebnost biofenolov in tokoferolov in ugotovili, da daje 2-fazni ekstrakcijski proces (DP) olja, ki imajo višjo vsebnost tokoferolov in biofenolov ter da količina dodane vode pri procesu DP znatno vpliva na vsebnost omenjenih antioksidantov. Nenazadnje pa smo tudi preučili vpliv neustreznega skladiščenja (toplota in svetloba) na vsebnost biofenolov in tokoferolov v nekaterih vzorcih deviških oljčnih olj letnikov 1997/98 in 1998/99 ter pokazali, da (neposredni) vpliv svetlobe znatno pospeši razpad tokoferolov in biofenolov.*

Ključne besede: deviško oljčno olje, biofenoli, tokoferoli, HPLC, predelava, Slovenska Istra

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MIKROSATELITSKI MARKERJI IN NJIHOVA UPORABNOST V OLJKARSTVU

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IZVLEČEK

Mikrosateliti se uporabljajo v genetskih raziskavah rastlin za študije raznolikosti, starševske analize, izdelavo genetskih kart in za genotipizacijo. Vsestranska uporabnost in popularnost mikrosatelitov temelji na pozitivnih lastnostih markerskega sistema: visoka pogostost pojavljanja v evkariotskih genomih, kodominatnost, hipervariabilnost, robustnost in visoka informacijska vrednost. Prvi mikrosateliti oljke so bili objavljeni v letu 2000 in od tedaj se pogosto vključujejo v različne genetske raziskave oljk. V delu so predstavljene karakteristike objavljenih mikrosatelitov oljke in njihova uporabnost v genetskih študijah.

Ključne besede: mikrosateliti, SSR, *Olea europaea* L., oljkarstvo, polimorfizem

MARCATORI MICROSATELLITARI E IL LORO IMPIEGO NELL'OLIVICOLTURA

SINTESI

I microsatelliti vengono utilizzati nelle ricerche genetiche delle piante, inerenti la diversità, l'analisi parentale, l'elaborazione di mappe genetiche e la genotipizzazione. L'impiego universale e la popolarità dei microsatelliti sono dovuti alle caratteristiche positive del sistema di marcatori: alta frequenza di presenza nei genomi eucariotici, codominanza, ipervariabilità, robustezza ed alto valore informativo. I primi microsatelliti dell'olivo sono stati pubblicati nel 2000 e da allora vengono di frequente inseriti in diverse ricerche genetiche degli olivi. Nell'articolo vengono presentate le caratteristiche dei microsatelliti dell'olivo pubblicati e il loro impiego negli studi genetici.

Parole chiave: microsatelliti, SSR, *Olea europaea* L., olivicoltura, polimorfismo

UVOD

Oljko že tisočletja gojijo na območju sredozemskega bazena, v glavnem zaradi pridobivanja oljčnega olja, ki ima izredno pomembno vlogo v človekovi prehrani. Zaradi ugodne sestave maščobnih kislin in vsebnosti številnih biološko pomembnih spojin uvrščamo oljčno olje med funkcionalna živila, za katerim povpraševanje na trgu nenehno narašča. Raziskave so pokazale, da so lastnosti oljčnega olja odvisne od sortne strukture oljk, h kakovosti in tipičnosti olja pa prispevajo tudi pedoklimatski dejavniki pridelovalnega območja in tehnologija predelave oljk. Sortna struktura je nastajala več stoletij in v literaturi je omenjenih več kot 1000 oljčnih sort, ki so se izoblikovale s selekcijskim pritiskom na agronomsko pomembne lastnosti ali spontano s križanjem gojenih in divjih oljk (Rugini & Baldoni, 2005). Izredna genska raznolikost oljk in pomanjkanje žlahtnjiteljskih programov, ki bi privedli do izboljšanja genskih virov, so glavni razlog pospešenega preučevanja genetske strukture oljk. Do razvoja molekulskih markerjev so raziskave genskih virov temeljile na uporabi klasičnih metod z opisovanjem morfoloških markerjev. Fenotipsko vrednotenje rastlinskega materiala je metodološko zapleteno, odvisnost morfoloških markerjev od dejavnikov okolja pa močno omejuje njihovo uporabo v genetskih študijah.

V zadnjih dvajsetih letih je razvoj številnih markerjev DNA omogočil revolucionarni pristop pri preučevanju strukture genoma in genske raznolikosti pomembnejših kmetijskih rastlin. Napredku molekularne biologije je sledilo tudi oljkarstvo, in markerji DNA se danes rutinsko uporabljajo za molekulsko karakterizacijo in identifikacijo oljčnih sort, v študijah izvora in domestikacije oljke, pri preučevanju genske variabilnosti divjih in kultiviranih oljk, identifikaciji markerjev, vezanih na agronomsko pomembne lastnosti, in pri genskem kartiranju (Bandelj *et al.*, 2002a). Večina razvitih markerskih sistemov je uporabnih tudi za genotipiziranje DNA oljk (RFLP, RAPD in AFLP). Kljub popularnosti teh tehnik pa je zanimanje med raziskovalci za novejše markerje, kot so mikrosateliti, vse večje. Z objavo tehnike mikrosatelitov (Litt & Luly, 1989) so ti markerji postali najbolj priljubljeni za molekulsko karakterizacijo različnih rastlinskih vrst (Gupta & Varshney, 2000). Le-ti združujejo lastnosti različnih markerjev in jih v literaturi pogosto predstavljajo kot idealen markerski sistem. Prvi mikrosateliti oljke so bili identificirani in predstavljeni šele v letu 2000 (Rallo *et al.*, 2000; Sefc *et al.*, 2000) in od tedaj se uspešno vključujejo v genetske raziskave oljk z različnimi cilji.

Namen pričujočega dela je podrobneje predstaviti mikrosatelitski markerski sistem, opisati karakteristike identificiranih mikrosatelitov oljke ter predstaviti njihovo uporabnost v genetskih raziskavah oljk.

MIKROSATELITI V RASTLINAH

Skupna lastnost evkariotskih genomov je ta, da vsebujejo tandemsko ponavljajočo se DNA, ki so jo poimenovali satelitna DNA. Odkrili so jo pri ultracentrifugiranju DNA v gradientnem mediju, ko se je le ta porazdelila v več plasti, med katerimi je plast vsebovala DNA, sestavljeno iz skupine tandemsko ponavljajočih se zaporedij. Satelitno DNA glede na dolžino osnovnega motiva delimo v tri skupine (Armour *et al.*, 1999):

1. sateliti: sestavljeni so iz osnovnega nukleotidnega zaporedja (motiva) dolžine do 200 baznih parov (bp), več takih ponovitev skupaj tvori satelit, ki ima lahko skupno dolžino nekaj magabaznih parov in predstavlja nekaj odstotkov genoma,
2. minisateliti: osnovni motiv je sestavljen iz več kot 10 nukleotidov, ki lahko dosežejo skupno dolžino od 0,5 do 30 kbp,
3. mikrosateliti: osnovni motiv je kratek (od 1 do 6 bp) in na posameznem lokusu tvori ponovitve skupne dolžine od 20 do 100 bp.

V literaturi se pojavljajo različna poimenovanja mikrosatelitov: STR (angl. Short Tandem Repeats – kratke tandemske ponovitve), SSR (angl. Simple Sequence Repeats – enostavna ponovljiva zaporedja), SSLP (angl. Simple Sequence Length Polymorphism – polimorfizem dolžin enostavnih zaporedij) in VNTR (angl. Variable Number of Tandem Repeats – spremenljivo število tandemskih ponovitev).

Glede na tip ponovitve osnovnega motiva, Chambers & MacAvoy (2000) predlagata naslednje poimenovanje mikrosatelitov:

– popoln mikrosatelit (angl. pure microsatellite) je sestavljen iz enega samega motiva baz, ki se tandemsko ponavlja in ni prekinjen z nobeno drugo bazo, npr. $(AC)_{14}$;
– sestavljen mikrosatelit (angl. compound microsatellite) sestavljata vsaj dva različna osnovna motiva ponovitve, npr. $-(CT)_{22}(CA)_6$;
– popoln in prekinjen mikrosatelit (angl. interrupted pure microsatellite) ima osnoven motiv prekinjen z insercijo enega nukleotida ali več baznih parov, ki se razlikujejo od osnovnega motiva, npr. $(CA)_4TA(CA)_7$;
– sestavljen in prekinjen mikrosatelit (angl. interrupted compound microsatellite) ima poleg vsaj dveh različnih osnovnih motivov še krajšo insercijo baznih parov, ki se razlikujejo od osnovnih motivov, npr. $(AC)_{14}AGAA(AG)_{12}$;
– kompleksen mikrosatelit (angl. complex microsatellite) je širši izraz za popolne in sestavljene mikrosatelite, ki nastanejo zaradi insercij baz, ki predstavljajo kratko ponovitev, npr. $(TC)_4(T)_6(CT)_4CTCC(TCC)_6$.

Poznana so tudi zaporedja DNA, ki ne vsebujejo ponovljivih motivov, ampak so nekakšno vmesno stanje, sestavljena iz več mešanih motivov, ki rahlo nakazujejo

na tandemsko ureditev. Ta zaporedja so poimenovali z izrazom prikrita enostavnost (angl. *cryptic simplicity*), za katere Hancock (1999) meni, da predstavljajo propadajočo mikrosatelitsko regijo, izpostavljeno številnim točkovnim mutacijam. Kriptične regije so lahko tudi mesto nastanka novih mikrosatelitov (Tautz *et al.*, 1986). Procesa izginjanja in nastajanja novega mikrosatelita potekata istočasno. Kratki mikrosateliti (imenovani tudi proto-mikrosateliti) nastajajo naključno, najprej pride do točkovnih mutacij, ki jim sledijo še redki zdrsi vijačnice med replikacijo (Levinson & Gutman, 1987).

Nestabilnost mikrosatelitskih lokusov in posledično velika variabilnost mikrosatelitskih markerjev sta prispevala k popularnosti markerskega sistema in njegove uporabe v mnogih evolucijskih in genetskih študijah. Velik polimorfizem je posledica sprememb v številu ponovitev osnovnega motiva, za kar sta odgovorna predvsem dva mehanizma, ki se verjetno dopolnjujeta (Eisen, 1999):

1. Model nepravilnega parjenja zdrsnjenih verig med replikacijo DNA (model SSM – Slip-Strand Mispairing) predvideva mutacijo, ki jo povzroči zdrs DNA v kompleksu s polimerazo, kar lahko povzroči nekomplementarnost matrične in nove sintetizirane verige. Če popravljalni mehanizmi napake zaradi zdrsa ne odpravijo, ostane poravnava obeh verig nepravilna. Mikrosatelitske ponovitve se zlahka izvijejo iz vijačnice v obliki zank. Nova sintetizirana veriga bo tako v naslednjih replikacijah spremenila svojo dolžino, pri mikrosatelitu se to kaže v nastanku ali izgubi ene ali več ponovitev.
2. Neenak *crossing-over* (model UCO – Unequal Crossing-Over) nastane kot rezultat rekombinacije med homolognima kromosomoma, ki nista bila popolno poravnana. Verjetnost nepravilne poravnave je zaradi mikrosatelitskih ponovitev velika.

Mutacijska stopnja vseh mikrosatelitskih lokusov ni enaka. Dognano je bilo, da na njen nivo vplivajo: število ponovitev in nukleotidno zaporedje osnovnega motiva, dolžina ponavljajoče se enote, DNA-zaporedje obrobnih regij, prekinitve v mikrosatelitu, stopnja rekombinacije in transkripcije. Raziskave mutacijske stopnje in evolucijske dinamike mikrosatelitov s številnimi odkritimi protislovji je nazorno povzel Schlötterer (2000).

Številne raziskave so pokazale, da se nukleotidna zaporedja minisatelitov in mikrosatelitov ne pojavljajo samo v nekodirajočih regijah genoma, kot je bilo prvotno mišljeno, temveč tudi v zgornjih promotorskih regijah kodirajočih DNA-zaporedij evkariotskih genomov, kjer imajo domnevno vlogo regulatornih elementov. Številne mikrosatelitske ponovitve, odkrite pri človeku, so bile najdene tudi pri primatih, kar nakazuje na njihovo biološko funkcionalnost. Mikrosateliti v promotorskih regijah kodirajočih zaporedij DNA delujejo kot ojačevalci ekspresijskih vektorjev. Znano je, da lahko odsotnost mikrosatelitske ponovitve zmanjša regulatorno sposobnost promotorja. V nadaljevanju so s študijami ugotovili, da lahko kratki nukleotidni in ponavljajoči se motivi v zgornjih aktivnih mestih ekspresijskih vektorjev služijo kot mesto vezave regulatornih proteinov. Predstavljene so bile tudi študije o vplivu dolžine mikrosatelitov na fenotipsko raznolikost (Kashi & Soller, 1999).

ZASTOPANOST MIKROSATELITOV PRI RASTLINAH

Podrobnejši pregled zastopanosti mikrosatelitov v genomu višjih rastlin sta med prvimi podala Morgante & Olivieri (1993), ki sta preučila pogostost pojavljanja dinukleotidnih in trinukleotidnih mikrosatelitskih ponovitev. Pri pregledu podatkovnih baz nukleotidnih zaporedij sta ugotovila, da se tip AT ponovitve najpogosteje pojavlja in sestavlja kar 74% vseh dinukleotidnih mikrosatelitov. Sledila mu je ponovitev GT/AC s 24-odstotno zastopanostjo, medtem ko je ponovitev AC/GT sestavljala le 1% vseh dinukleotidnih ponovitev. Dinukleotidne ponovitve CG avtorja nista zasledila. Med trinukleotidnimi ponovitvami sta bila motiva TAT in TCT najpogostejša. Pregled mikrosatelitov pri 34 različnih rastlinskih vrstah je pokazal, da so le ti v genomu pogosti in se pojavljajo na vsakih 50 kbp. V raziskavi pogostosti mikrosatelitov in tipov mikrosatelitskih ponovitev pri različnih evkariotskih genomih Tóth *et al.* (2000) odkrivajo, da tri- in heksanukleotidne ponovitve mikrosatelitov prevladujejo v eksonih, saj izguba ali pridobitev take ponovitve ne poruši bralnega okvirja. Pojavljanje drugih tipov mikrosatelitov v medgenskih regijah in intronih pa je taksonomsko specifično. O podobnih izsledkih poročajo še Metzgar *et al.* (2000). V nekodogenih regijah obstajajo mono-, di- tri-, tetra- in heksanukleotidni mikrosateliti, za katere veljajo podobni mutacijski in selekcijski procesi. V kodogenih regijah se v večji meri pojavljajo tri- in heksanukleotidni mikrosateliti, ki so izpostavljeni močnejšim in bolj specifičnim selekcijskim pritiskom. V novejši raziskavi so Morgante *et al.* (2002) preučili zastopanost mikrosatelitskih ponovitev v genomskih in EST (Expressed Sequence Tags – izražena nukleotidna zaporedja) zaporedjih repnjakovca (*Arabidopsis thaliana*), riža, soje, koruze in pšenice. Odkrili so, da je frekvenca pojavljanja mikrosatelitov obratno sorazmerna z velikostjo genoma in deležem ponavljajoče se DNA, in da ostaja konstantna v kodogenem delu genoma. Mikrosateliti so večinoma pojavljajo v regijah, ki predstavljajo nedavno povečanje genoma pri rastlinah, njihova frekvenca je večja v prepisanih regijah, posebno v neprevedenem delu mRNA, kjer je lahko večja celo za 3-krat. Pri vseh rastlinah, ki so bile vključene v analizo, pa je frekvenca mikrosatelitov znatno večja v knjižnici EST, kjer so odkrili najpogostejšo ponovitev AG/CT in najmanjšo AT. Song *et al.* (2002) so ugotovili, da je med trinukleotidni mikrosateliti najpogostejši in tudi najbolj polimorfen pri pšenici motiv TAA. V nadaljevanju McCouch *et al.* (2002) poročajo o zastopanosti mikrosatelitov pri rižu, kjer je najpogostejši

motiv GA, sledita mu motiva AT in CCG. Najdaljši so bili mikrosateliti z motivom AT, najkrajši pa s ponovitvijo GA.

TEHNIKA NAMNOŽEVANJA MIKROSATELITSKIH LOKUSOV

Princip tehnike mikrosatelitskih markerjev se od tehnik AFLP in RAPD razlikuje v tem, da se v verižni reakciji s polimerazo namesto naključnih lokusov namnožujejo znani lokusi. Če je znano nukleotidno zaporedje obrobne regije mikrosatelita, se lahko izdela lokusno specifična začetna oligonukleotida, običajno dolžine 18–25 bp, ki omogočata namnožitev mikrosatelitske regije. Namnoženi aleli se ločijo z elektroforezo v poliakrilamidnem gelu ali polimeru visoke ločljivosti, za zaznavanje mikrosatelitov pa se uporablja radioaktivnost ali barvanje s srebrom. Popularna je tudi uporaba fluorescentno označenih začetnih oligonukleotidov in zaznavanje namnoženih alelov z avtomatsko lasersko napravo, ki omogoča hitro nadaljnjo obdelavo rezultatov (Kozjak *et al.*, 2003b; Štajner *et al.*, 2005). Fluorescentno označevanje molekul DNA običajno zviša ceno analiz, vendar je danes na voljo tehnika ekonomičnega fluorescentnega označevanja molekul PCR (Schuelke, 2000), ki je bila preizkušena in uspešno uporabljena pri namnoževanju in zaznavanju mikrosatelitov kač (Scott et al., 2001) in oljke (Bandelj *et al.*, 2004a).

LASTNOSTI MIKROSATELITOV IN UPORABNOST V GENETSKIH ŠTUDIJAH

Mikrosateliti združujejo lastnosti različnih markerjev, zaradi česar so izredno atraktivni za genetske študije rastlin. Poleg visoke pogostosti pojavljanja in enakomerne razpršenosti v evkariotskih genomih jih odlikujejo še kodominantna narava, hipervariabilnost, visoka stopnja polimorfizma ter informativnost (Morgante & Olivieri, 1993; Powell *et al.*, 1996a; Weising *et al.*, 1998). Prednost uporabe mikrosatelitskih markerjev je tudi v možnosti avtomatiziranja postopka. Robustnost jim pripisujejo zaradi dobre ponovljivosti rezultatov (Powell *et al.*, 1996b; Jones *et al.*, 1997).

Rezultati genotipizacije rastlin z mikrosateliti so med laboratoriji primerljivi, nekaj odstopanj pri dolžini alelov pa je vendarle možno zaslediti zaradi različnih postopkov ločevanja in detekcije namnoženih markerjev (Weber, 1990). Bowers *et al.* (1996) so pri primerjavi dolžin alelov zaznanih s srebrom in dolžin avtomatskega fluorescentnega zaznavanja odkrili razlike v velikosti od 1 do 2 bp. Podobno ugotavljajo tudi Kozjak *et al.* (2003a), ki so pri genotipizaciji klonov vinske trte kultivarja 'Refošk' na dveh mikrosatelitskih lokusih pri barvanju s srebrom odkrili 2 bp daljša alela v primerjavi z avtomatskim fluorescentnim zaznavanjem na laserski napravi ALFexpress. Kline *et al.* (1997) so ugotavljali primerljivost rezultatov mikrosatelitske analize v 34 laboratorijih in odkrili, da so rezultati primerljivi, če alele poimenujejo opisno. Pri primerjavi alelov, ki so jih označili z dolžino, je prihajalo do razlik, večjih od 5 bp.

Poleg različnih elektroforetskih sistemov in postopkov zaznavanja alelov so manjša neskladja v dolžini alelov lahko tudi posledica pojava senčnih fragmentov. Weber & May (1989) pojav razlagata kot posledico zdrsa polimeraze *Taq* in matrične vijačnice med namnoževanjem DNA v verižni reakciji s polimerazo. Murray *et al.* (1993) so z določitvijo nukleotidnega zaporedja senčnih fragmentov dinukleotidnega mikrosatelitskega lokusa ugotovili, da ti predstavljajo delecijo 2 bp, kar ustreza dolžini ene mikrosatelitske ponovitve. Pojav je izrazitejši pri dinukleotidnih mikrosatelitih in lahko povzroči nepravilno določitev dolžine alelov.

Med slabše lastnosti mikrosatelitskega markerskega sistema raziskovalci uvrščajo visoke stroške izolacije novih markerjev (Rafalski & Tingey, 1993; Squirrell *et al.*, 2003). Tradicionalne postopke, ki vključujejo izdelavo delne genomske knjižnice, kloniranje in hibridizacijsko preverjanje velikega števila rekombinantnih klonov, zamenjujejo novejše tehnike, ki vključujejo izdelavo genomske knjižnice, obogatene z mikrosateliti (Jakše & Javornik, 2001). Uspešnost izolacije mikrosatelitov je odvisna od več korakov, od priprave genomske knjižnice pa vse do izdelave parov začetnih oligonukleotidov, ki imajo sposobnost namnoževanja specifičnih lokusov s polimorfnimi aleli (Squirrell *et al.*, 2003).

Ko so mikrosateliti za določeno vrsto že poznani, se lahko uporabijo tudi za genetske analize sorodnih vrst, kar zniža stroške analiz, saj izdelava genomskih knjižnic v tem primeru ni potrebna. Obrobna nukleotidna zaporedja mikrosatelita so lahko med sorodnimi vrstami ohranjena, zato nekateri začetni oligonukleotidi, pripravljeni za eno vrsto, uspešno namnožujejo tudi domnevno ortologno regijo DNA druge vrste. Namnoženi produkti PCR pričakovane dolžine ne zagotavljajo nujno obstoj mikrosatelitov in dovolj velikega polimorfizma (Huang *et al.*, 1998). Peakall *et al.* (1998) so z določitvijo nukleotidnega zaporedja dognali, da so razlike med aleli različnih vrst kompleksnejše, saj se ne razlikujejo samo v spremembi števila ponovitev osnovnega motiva, temveč tudi v strukturi mikrosatelita. Uspešnost medvrstne uporabe mikrosatelitskih markerjev se zmanjšuje s povečevanjem filogenetske oddaljenosti vrst (Schlötterer, 1998).

Uporaba začetnih oligonukleotidov sorodnih vrst za namnoževanje mikrosatelitskih regij je lahko povezana tudi s pojavom ničtih alelov (izpad namnožitve alela). Za nastanek ničtih alelov so odgovorne mutacije na mestih prileganja začetnih oligonukleotidov, ki preprečijo vezavo začetnih oligonukleotidov in s tem namnožitev mikrosatelita. Posledica so spremenjene frekvence alelov in genotipov, kar lahko privede do presežka homozigotov in podcenitve heterozigotnosti preučeva-

Tab. 1: Kronološka predstavitev genetskih raziskav oljke, pri katerih so bili uporabljeni mikrosatelitski markerji.
Tab. 1: Chronological presentation of genetics investigations of olives with the aid of microsatellite markers.

Cilji raziskave	Opis raziskave	Referenca
Izolacija in karakterizacija mikrosatelitov	Identifikacija in karakterizacija 15-ih MS lokusov oljke (ssrOeUA-DCA).	Sefc *et al.*, 2000
Izolacija in karakterizacija mikrosatelitov	Identifikacija in karakterizacija 5-ih MS lokusov oljke (IAS-oli).	Rallo *et al.*, 2000
Genotipizacija oljčnih sort	Preučili identiteto dveh sort iz IT in CA (Oblonga in Frantoio) s 5 MS lokusi. Ugotovili identičnost sort na vseh lokusih.	Barranco *et al.*, 2000
Izolacija in karakterizacija mikrosatelitov	Razvili 20 novih MS markerjev in predstavili karakterizacijo lokusov na 16-ih sortah (GAPU).	Carriero *et al.*, 2002
Izolacija in karakterizacija mikrosatelitov	Identifikacija 30 MS lokusov in karakterizacija lokalnih oljčnih sort v Italiji (UDO).	Cipriani *et al.*, 2002
Izolacija in karakterizacija mikrosatelitov	Identificirali 7 MS lokusov (EMO).	De la Rosa *et al.*, 2002
Genotipizacija oljčnih sort z mikrosatelitskimi markerji	Genotipizirali oljčne sort iz nacionalnega kolekcijskega nasada. Za genotipizacijo uporabili 14 MS lokusov.	Bandelj *et al.*, 2002b
Primerjalna študija RAPD, AFLP in MS tehnik pri identifikaciji in ugotavljanju sorodnostnih odnosov oljčnih sort	Preučili informativnost posameznega markerskega sistema. V analizo vključili 32 sort. Uporabili 8 predhodno objavljenih MS lokusov.	Belaj *et al.*, 2003
Izdelava genetske karte	Za kartiranje uporabili RAPD, AFLP, RFLP in MS markerje. Uporabili MS lokuse bližnjih sorodnikov oljke.	De la Rosa *et al.*, 2003
Namnoževanje MS oljke pri sorodnih vrstah znotraj rodu *Olea*	S 4-mi MS lokusi dosegli uspešno namnoževanje MS pri 13-ih različnih vrstah in podvrstah rodu *Olea*. Ugotovili visok polimorfizem in preučili sorodnost med vrstami.	Rallo *et al.*, 2003
Vrednotenje genetske raznolikosti in preučevanje sorodnosti oljčnih sort	Preučili 19 oljčnih sort z uporabo 14-ih MS lokusov. Predstavili informacijske vrednosti lokusov.	Bandelj *et al.*, 2004b
Izdelava integrirane genetske karte	190 markerjev (MS, RAPD, SCAR) uporabili za konstrukcijo karte, od tega 10 MS markerjev.	Wu *et al.*, 2004
Aplikacija MS za preverjanje pristnosti ekstra deviških oljčnih olj	Izolacija DNA iz komercialnih oljčnih olj in namnoževanje MS. Primerjava genetskih profilov sort z aleli, odkritih v oljčnem olju. Poročanje o uspešnosti metode MS v "forenziki" oljčnih olj.	Breton *et al.*, 2004
Klonska variabilnost	Analizirali variabilnost 130-ih vzorcev oljk. Genotipizacijo opravili na 14-ih MS lokusih. Pri več kot 60% vzorcih ugotovili homonime ali napačno označitev dreves v kolekciji in ugotovili visoko stopnjo variabilnosti znotraj oljčnih sort.	Lopes *et al.*, 2004
Starševska analiza	Uporabili 8 MS za genotipizacijo 23 oljčnih sort, potencialnih za uporabo v žlahtnjiteljskih programih. 4 MS uporabili za testiranje križancev.	De la Rosa *et al.*, 2004
Genotipizacija oljčnih sort in preučevanje genetske raznolikosti oljk	Preučili 46 akcesij 30-ih sort iz Sicilije (IT). V analizo vključili 12 MS lokusov.	La Mantia *et al.*, 2005
Preučevanje sorodnostnih odnosov in identifikacija sort	Preučenih 111 akcesij 60-ih oljčnih sort z markerji AFLP in MS. Uporabili 27 MS lokusov, izdelali dendrogram in preučili sposobnost ločevanja sort z dvema markerskima sistemoma.	Montemurro *et al.*, 2005
Aplikacija MS za preverjanje pristnosti ekstra deviških oljčnih olj	Izolacija DNA iz komercialnih oljčnih olj in namnoževanje MS. Uporabili 6 MS lokusov, izdelali začetne oligonukleotide za ugnezden PCR, uspešno namnoževanje MS, daljših od 188 bp.	Testolin & Lain, 2005
Upravljanje kolekcije oljk in identifikacija sort v Italiji	Preučili raznolikost 39 akcesij oljk iz Apulie (IT). Mikrosatelitom določili nukleotidno zaporedje in vsako sorto opisali s številom ponovitev osnovnega motiva MS. Uporabili 5 MS lokusov.	Muzzalupo *et al.*, 2006

nega lokusa. Obstoj ničtih alelov se lahko potrdi le s segregacijsko analizo. Pojavu se lahko izognemo z izdelavo novih specifičnih začetnih oligonukleotidov na novih mestih obrobnih zaporedij, če je to mogoče.

Za markerje, ki se uporabljajo na nivoju kromosomov pri kartiranju genoma, je pomembno, da so številčni in da se enakomerno pojavljajo v genomu. Tipičen mikrosatelitski lokus izpolnjuje oba kriterija, zato so mikrosateliti idealno orodje za kartiranje genoma. Zelo učinkoviti so tudi pri kartiranju lokusov, vezanih na kvantitativne lastnosti (QTL, Quantitative Trait Locus) (Chambers & MacAvoy, 2000).

Mikrosateliti so zaradi hipervariabilnosti idealno orodje tudi za molekulsko identifikacijo posameznikov. Vsak posameznik ima svojevrsten vzorec alelov, ki je osebno specifičen. Tehnika genotipizacije se rutinsko uporablja v sodno medicinskih raziskavah pri prepoznavanju oseb in ugotavljanju sorodstvenih vezi (Zupančič, 1998). Tudi pri rastlinah so mikrosateliti primerni za genotipizacijo in identifikacijo sort, kultivarjev, klonov in akcesij. S pomočjo mikrosatelitov se ugotavljajo nepravilnosti pri poimenovanju sort, ki so zaradi sinonimov in homonimov pogoste pri vegetativno množenih rastlinah.

Mikrosateliti so bili v rastlinski genetiki uspešno uporabljeni tudi pri ugotavljanju genetske sorodnosti. Ker se dedujejo kodominantno, so idealno orodje za starševske analize in analize rodovnikov. S pomočjo mikrosatelitov se pridobivajo informacije o žlahtnjenju rastlin, o nastanku in strukturi populacij rastlin ter njihovi domestikaciji. Kronološki pregled raziskav o razvoju mikrosatelitskega markerskega sistema in njegove aplikacije v molekularno-genetskih raziskavah pri oljki je predstavljen v Tabeli 1.

PREDSTAVITEV KARAKTERISTIK OBJAVLJENIH MIKROSATELITOV OLJKE

Med letoma 2000 in 2006 je 5 raziskovalnih skupin poročalo o identifikaciji 77-ih mikrosatelitskih lokusov. Sefc *et al.* (2000) so pregledali genomsko knjižnico s sondami GA in CA in identificirali 20 mikrosatelitov z motivom GA, 4 z motivom CA in 5 s sestavljenim motivom CA-GA. Začetne oligonukleotide so izdelali za 28 lokusov, mikrosatelite pa uspešno namnožili na 15-ih lokusih. Karakterazijo lokusov so predstavili v skupini 38 sort oljk iz Španije in 9 iz Italije.

Lokusi serije ssrOeUA-DCA so bili med prvimi objavljenimi, zato so bili tudi največkrat uporabljeni v genetskih študijah oljk. Molekulska karakterizacija oljčnih sort na 14-ih lokusih DCA je bila opravljena v Sloveniji (Bandelj *et al.*, 2004b) in na Portugalskem (Lopes *et al.*, 2004). Število namnoženih mikrosatelitov se med študijami razlikuje, saj je bilo v analize vključeno različno število sort različnega geografskega izvora. V vseh treh študijah (Sefc *et al.*, 2000; Bandelj *et al.*, 2004b; Lopes *et al.*, 2004) je bila opažena heterozigotnost nižja od pričakovane na lokusih DCA4, DCA11 in DCA13, kar nakazuje na možnost obstoja ničtih alelov. Najvišja opažena heterozigotnost je bila ugotovljena na lokusih DCA3, DCA8, DCA9, DCA14, DCA16 in DCA18. V povprečju je bila opažena heterozigotnost v treh raziskavah visoka (0,722), kar kaže na veliko genetsko variabilnost oljk. Zohary & Spiegel-Roy (1975) menita, da so gojeni kloni oljk ekstremno heterozigotni. Pred domestikacijo so se oljke množile spontano s križanji, kar je bil razlog za povečano heterozigotnost, ki je rastlinam omogočila preživetje. V določenem obdobju pa je generativno razmnoževanje prešlo v vegetativno, tako da je heterozigotnost ostala fiksirana.

Informacijska vrednost polimorfizma (PIC vrednost) je odvisna od števila alelov in njihove frekvence na posameznem lokusu (Botstein *et al.*, 1980). Na osnovi povprečne vrednosti PIC (0,675) je bilo ugotovljeno, da so mikrosatelitski lokusi serije DCA zelo informativni. Med zelo informativne (PIC > 0,5) so se uvrstili vsi lokusi razen DCA5 in DCA13, 8 lokusov (DCA3, DCA4, DCA7, DCA9, DCA10, DCA14, DCA16 in DCA17) pa je izpolnjevalo tudi kriterij o primernosti lokusa za kartiranje (PIC > 0,7) (Bandelj *et al.*, 2004b).

Na lokusih DCA4 in DCA14 je bilo ugotovljeno kompleksno namnoževanje mikrosatelitov in odmik od pričakovanega dialelnega elektroforetskega vzorca, ki je običajen za diploidne organizme (Bandelj *et al.*, 2004b). Odmik se je pokazal v namnoževanju tretjega alela, kar je lahko posledica namnoževanja dodatnega lokusa. Ancestralne duplikacije kromosomov in poliploidni ali alopoliploidni značaj rastlinske vrste so po navedbah iz literature največkrat vzrok večlokusnega namnoževanja mikrosatelitov. Pri oljki Cipriani *et al.* (2002) poročajo, da 17% začetnih oligonukleotidov na novo izoliranih mikrosatelitov verjetno namnožuje dva različna lokusa. Avtorji menijo, da visoka frekvenca podvojenih DNA regij nakazuje na možnost v celoti podvojenega genoma, vendar njihovi rezultati ne zadostujejo, da bi ugotovili, ali je oljka poliploidnega ali alopoliploidnega značaja.

Pojav dominance kratkega alela, ki se kaže v preferenčnem namnoževanju kratkih alelov (Wattier *et al.*, 1998), je bil opažen na lokusih DCA1, DCA10 in DCA17. Pri heterozigotnih genotipih, kjer sta bila navzoča kratek in dolg alel, je bila po barvanju s srebrom opažena večja intenziteta krajšega alela v primerjavi z daljšim, kar je verjetno posledica kompeticije namnoževanja kratkih in dolgih alelov v verižni reakciji s polimerazo (Bandelj *et al.*, 2004b). Selektivno namnoževanje alelov na takih lokusih lahko privede do podcenitve opažene heterozigotnosti in posledično vpliva na rezultate genetske analize.

Z obogatitvenim postopkom genomske knjižnice oljke z motivom GA so Rallo *et al.* (2000) z radioaktivno označeno sondo identificirali 24 klonov z mikrosatelitom. 55% klonov je vsebovalo popolne mikrosatelite,

30% nepopolne, pri 15% pa je bil ugotovljen sestavljen motiv mikrosatelita. Odkrili so mikrosatelitske motive CA, TA, GAA v kombinaciji z GA v sestavljenem mikrosatelitu, v enem primeru pa poročajo tudi o heksanukleotidnem motivu AGAGGG. Lokusno specifične oligonukleotide so pripravili za 13 mikrosatelitskih regij. Namnoževanje mikrosatelitov so preizkusili na 46 oljčnih sortah različnega geografskega izvora in ugotovili, da le 5 lokusov namnoži polimorfne produkte PCR v pričakovanem velikostnem območju (IAS-oli06, IAS-oli11, IAS-oli12, IAS-oli17, IAS-oli22). Na dveh lokusih niso dosegli namnoževanja alelov, pri 4 so ugotovili kompleksno in nespecifično namnoževanje, dva lokusa pa sta bila monomorfna. Skupno število namnoženih alelov v skupini 46 oljčnih sort na 5-ih mikrosatelitskih lokusih je bilo 26. Največ alelov (9) so ugotovili na lokusu IAS-oli11, najmanj (4 in 3) pa na lokusih IAS-oli17 ter IAS-oli06 in IAS-oli22. Lokusi z manjšim število alelov so zaradi večje verjetnosti identičnosti genotipov manj primerni za identifikacijo sort. Dedovanje mikrosatelitov so preverili z genotipizacijo potomcev križanja 'Leccino' X 'Dolce Agogia' in potrdili Mendlovsko dedovanje. Pri karakterizaciji izoliranih mikrosatelitov avtorji poročajo o pojavu ničtih alelov, senčnih fragmentov in kompleksnega namnoževanja lokusov. Na lokusu IAS-oli12 so odkrili ničte alele, ki so verjetno posledica mutacij na mestih prileganja začetnih oligonukleotidov. Namnoževanje nespecifičnih produktov PCR so ugotovili na lokusu IAS-oli08, zato so ga izključili iz nadaljnjih analiz. Pojav senčnih fragmentov, ki so bili izrazitejši pri zaznavanju alelov z avtomatizirano sekvenčno napravo ABI 310, so avtorji opazili na lokusu IAS-oli11.

Genomsko knjižnico oljke, obogateno z motivom GA, so pripravili tudi Carierro *et al.* (2002). Avtorji so identificirali 54 pozitivnih klonov, med katerimi je 22 vsebovalo popolne dinukleotidne mikrosatelite, 15 nepopolne in 5 popolne trinukleotidne mikrosatelite. Preostalih 12 klonov so klasificirali med kompleksne mikrosatelite. Začetne oligonukleotide so izdelali za 20 mikrosatelitskih lokusov in na 10-ih dosegli uspešno namnoževanje polimorfnih alelov v skupini 16-ih sort oz. 20-ih akcesij. Na preostalih lokusih niso ugotovili polimorfnih markerjev. Najmanj alelov so odkrili na lokusih GAPU12 (2) in GAPU11e17 (3). Sledila sta jima lokusa GAPU45 in GAPU59 s štirimi aleli. Največ alelov so ugotovili na lokusu GAPU101 (9), sledili so mu lokusi GAPU103A, GAPU47 in GAPU89 z 8 aleli. Skupno število namnoženih alelov pri 20-ih akcesijah oljk je bilo 57, v povprečju 5,7 alela na lokus. S predstavljenimi mikrosateliti so avtorji preučili genetsko sorodnost oljk italijanskega izvora, o natančnejši informacijski vrednosti mikrosatelitov pa niso poročali.

Cipriani *et al.* (2002) so določili nukleotidno zaporedje 60 pozitivnih kolonij dveh obogatenih genomskih knjižnic (AC/GT in AG/CT) oljke. Na 30 mikrosatelitskih lokusih so dosegli uspešno namnoževanje alelov v pričakovani dolžini. Karakteristike mikrosatelitov so preučili na 12-ih sortah oljk italijanskega izvora. 28 mikrosatelitskih lokusov je bilo polimorfnih, na dveh pa polimorfizma niso ugotovili (UDO99-003, UDO99-022). Z začetnimi oligonukleotidi UDO99-007, UDO99-009, UDO99-022, UDO99-034, UDO99-036 sta se istočasno namnožila po dva lokusa. V povprečju so na lokusu odkrili po 3 alele, kar je v primerjavi z drugimi objavljenimi mikrosateliti oljke razmeroma malo. Na 7 lokusih sta bila ugotovljena le po 2 alela, na 9 lokusih so odkrili po 3 alele, na 3 lokusih 4 oz. 5 alelov. Kljub nizkemu številu namnoženih mikrosatelitov v 12-ih sortah oljk so avtorji analizirane sorte zlahka ločili. Podrobneje so preučili in primerjali sorti 'Casaliva' in 'Frantoio' ter 'Les' in 'Leccino'. Na osnovi genotipizacije večjega števila vzorcev sort 'Casaliva' in 'Frantoio' avtorji menijo, da 'Casaliva' pripada sortni populaciji 'Frantoio', vendar sta sorti genetsko različni. Enako velja tudi za sorti 'Leccino' in 'Les'.

De la Rosa *et al.* (2002) so predstavili še 7 novih mikrosatelitskih lokusov (EMO), ki so jih identificirali iz knjižnice, obogatene z dinukleotidnimi motivi GA in GT. Začetne oligonukleotide so izdelali za 13 mikrosatelitskih lokusov, vendar s 5-imi niso namnožili alelov v pričakovani dolžini, 2 lokusa pa sta bila monomorfna. Karakterizacijo novih mikrosatelitov so predstavili v skupini 23-ih sort. V povprečju so odkrili 6,4 alela na lokus, najmanj na lokusu EMOL (2), največ pa na lokusu EMO2 (9). Informacijsko vrednost polimorfizma lokusov so podali tudi z izračunom pričakovane in opažene heterozigotnosti. Najnižjo vrednost so opazili na lokusu z najmanjšim številom alelov EMOL. Z začetnimi oligonukleotidi lokusa EMO30 so pri sorti Arbequina namnožili 4 alele, kar nakazuje na istočasno namnožitev več lokusov. Uspešnost namnoževanja oljčnih mikrosatelitov so ugotovili tudi pri sorodnih vrstah oljke; forziciji (*Forsythia intermedia*), velikem jesenu (*Fraxinus excelsior*), jasminu (*Jasminum beesianum*), osmantu (*Osmanthus heterophyllus*) in španskem bezgu (*Syringa vulgaris*).

UPORABNOST MIKROSATELITOV V GENETSKIH ŠTUDIJAH OLJK

Identifikacija in genotipizacija oljčnih sort

Enostavno, vegetativno razmnoževanje oljke je omogočilo intenzivno izmenjavo rastlinskega materiala v državah Sredozemlja, kar je povzročilo veliko zmedo pri imenovanju sort in klonov. Sinonimi in homonimi pomenijo oviro pri vrednotenju genskih virov oljke, zato je karakterizacija genotipov z molekulskimi markerji najbolj primeren način za pravilno identifikacijo sort, ki je ključnega pomena pri upravljanju kolekcij, ločevanju sadilnega materiala v drevesnicah in pri vzgoji certificiranih sadik sort in klonov. Zaradi enostavnega vrednotenja alelnih profilov in zagotavljanja ponovljivosti

rezultatov med laboratoriji so mikrosateliti najprimernejše orodje za ločevanje sort.

Prvo obsežnejše delo na področju identifikacije oljčnih sort v Sloveniji je bilo prikazano v delu Bandelj *et al.* (2002b). V raziskavi je bilo določeno najmanjše število mikrosatelitov serije ssrOeUA-DCA (Sefc *et al.*, 2000), s katerimi je bilo mogoče ločiti 19 oljčnih sort iz nacionalnega kolekcijskega nasada oljk v Strunjanu. Najprimernejši markerji so bili izbrani na osnovi naslednjih kriterijev: namnoževanje nekompleksnih elektroforetskih vzorcev s kakovostnimi PCR produkti, stabilna struktura mikrosatelita in visoka informacijska vrednost markerjev. Verjetnost enakosti genotipov (PI), ki se uporablja kot merilo ločevanja genotipov, je pokazala nizko informacijsko vrednost lokusov DCA1, DCA5, DCA13 in DCA15. Pri omenjenih lokusih so bile vrednosti PI višje, zato jih uvrščamo med markerje s slabšo sposobnostjo ločevanja in so manj primerni za identifikacijo. Največja sposobnost ločevanja genotipov oljk je bila odkrita pri lokusu DCA16, kjer je bila vrednost PI najnižja (0,073). Na tem lokusu je bilo ugotovljenih 9 različnih alelov, največ efektivnih alelov (6,6) in največ različnih genotipov (14). Visoko informacijsko vrednost lokusa dokazuje tudi visoko število svojevrstnih genotipov (11). Dobro sposobnost ločevanja posameznikov je imel tudi lokus DCA10 z vrednostjo PI 0,078 in na katerem je bilo odkritih največ sortno specifičnih alelov (7), ki omogočajo takojšnjo identifikacijo specifičnega genotipa. Med preostalimi lokusi je bil glede na število ugotovljenih edinstvenih genotipov (8) in kakovost PCR produktov za identifikacijo izbran še lokus DCA3. Na osnovi upoštevanih kriterijev so bili med 14-imi mikrosatelitskimi lokusi za identifikacijo izbrani DCA3, DCA10 in DCA16, katerih kombinacija je zagotavljala svojevrstne alelne profile, karakteristične za specifično sorto.

Barranco *et al.* (2000) so preučili uporabnost mikrosatelitskih markerjev pri ugotavljanju sinonimov sort. V analizo so vključili sorti 'Oblonga' iz Kalifornije in 'Frantoio' iz Italije. Pri morfološki karakterizaciji obeh sort v genski banki so odkrili, da sta sorti fenotipsko identični, zato so ju primerjali z mikrosatelitskimi markerji. Identičnost alelnih profilov so odkrili na petih lokusih (IAS-oli06, IAS-oli11, IAS-oli12, IAS-oli17, IAS-oli22). Na osnovi rezultatov molekulske in morfološke analize so potrdili, da sta sorti genotipsko identični.

Rallo *et al.* (2000) so s 5-imi lokusi (IAS-oli) ločili 95% od 46 analiziranih sort. Svojevrstne alele, ki omogočajo takojšnjo identifikacijo, so zaznali na dveh lokusih pri 3 sortah, kar je v primerjavi s serijo mikrosatelitov ssrOeUA-DCA, kjer je bilo ugotovljenih kar 25 sortno specifičnih alelov pri 10-ih sortah (Bandelj *et al.*, 2002b), razmeroma malo. Slednje potrjuje visoko informacijsko vrednost lokusov serije ssrOeUA-DCA. De la Rosa *et al.* (2002) ravno tako poročajo o dobri ločevalni sposobnosti mikrosatelitov serije EMO, saj so s 4-mi lokusi (EMO2, EMO3, EMO13 in EMO30) ločili vseh 23 oljčnih sort.

Preučevanje genetske variabilnosti in sorodnosti oljk z mikrosateliti

Inventarizacija in ohranjanje genskih virov oljk ostajata prioriteti sodobnega oljkarstva. Poznavanje genetske variabilnosti je pomembno pri načrtovanju žlahtnjenja oljke, saj so pomanjkljive informacije o genskih virih glavni razlog, da oljka v preteklosti ni bila vključena v večje žlahtnjiteljske programe. Z namenom pospeševanja vzgoje sort, ki bi bile bolje prilagojene sodobnim agronomskim tehnologijam, v državah Sredozemlja ustanavljajo nacionalne in mednarodne kolekcije, kjer potekajo postopki vrednotenja in identifikacije genetskega materiala oljke.

Za ohranitev genskih virov oljke na Siciliji je bil v letu 1995 vzpostavljen kolekcijski nasad. Na osnovi predhodno objavljenih karakteristik mikrosatelitskih markerjev so La Mantia *et al.* (2005) napravili raziskavo genetske raznolikosti 46-ih vzorcev oljk, ki pripadajo skupini 30-ih sort. Genotipizacijo vzorcev so opravili na 12-ih mikrosatelitskih lokusih (DCA3, DCA4, DCA9, DCA16, GAPU101, GAPU59, UDO-008, UDO-009, UDO-012, UDO-024, UDO-039, UDO-043), izbranih na osnovi sposobnosti odkrivanja visokega polimorfizma, odsotnosti pojava senčnih fragmentov in nekompleksnega namnoževanja alelov. Z izbranimi lokusi so v skupini analiziranih oljk namnožili 119 mikrosatelitov, v povprečju so ugotovili 9,5 markerja na lokus. Za lokus UDO-009 je bila ugotovljena nizka sposobnost ločevanja genotipov zaradi majhnega števila namnoženih markerjev in visoke frekvence ponavljanja treh alelov v skupini oljk. Pri tem lokusu so ugotovili tudi pojav fragmenta +1 bp, kar je preprečevalo pravilno določitev dolžine alelov. Na lokusih UDO-043 in DCA4 so opazili značilne vzorce zdrsnjenih alelov, pojav ničtih aleov pa je bil ugotovljen na lokusih UDO-008 in UDO-039. Pri več kot 16-ih vzorcih so ugotovili sinonime. Na osnovi izračunanih koeficientov sorodnosti oljk so izdelali dendrogram in poskušali ugotoviti, ali se oljke s Sicilije genetsko razlikujejo od sort, ki uspevajo v drugih območjih Sredozemlja. Razmestitev sort v skupine ni pokazala večje genetske sorodnosti med oljkami glede na fenotipsko podobnost in geografsko območje gojenja, kar avtorji pojasnjujejo kot posledico prenašanja rastlinskega materiala oljk med Sicilijo in drugimi sredozemskimi državami. Z analizo so ugotovili tudi starševstvo dveh sort. Sorta 'Giarfara' je nastala s križanjem sort 'Nocellara del Belice' in 'Cacaridduni', medtem ko je sorta 'Pizzo di Corvo' križanec sort 'Nocellara Etnea' in 'Tonda Iblea'.

Avtorji mikrosatelitov GAPU (Carriero *et al.*, 2002) so markerje uporabili za preučitev genetske podobnosti 20 vzorcev oljk iz južne (Apulija, Kalabrija, Bazilika) in centralne Italije. Na osnovi izračunanih koeficientov podobnosti so z metodo UPGMA izdelali dendrogram, v katerem so bile sorte razdeljene v dve večji skupini. V prvi so se združile sorte, ki uspevajo na obali Ionskega

morja, druga skupina pa je bila razdeljena v dve manjši podskupini: kalabrijsko in apulijsko. Avtorji ugotavljajo, da so se sorte razvrstile v sorodnostne skupine glede na geografski izvor oljk.

V Sloveniji so bili mikrosateliti uporabljeni za ugotavljanje genetske sorodnosti oljčnih sort, ki uspevajo na območju Istre (Bandelj *et al.*, 2004b). Z molekulsko raziskavo smo poskušali ugotoviti genetsko povezavo lokalnih sort z italijanskimi, saj je študija oljčne strukture konec 19. in v začetku 20. stoletja pokazala, da je sortiment oljk na območju Istre nastajal pod vplivom Italije, zaradi priseljevanja ljudi iz osrednje Italije na območje Beneške republike (Hugues, 1999). Podobnost je Hugues (1999) opazil tudi v poimenovanju sort. Za analizo so bili uporabljeni lokusi serije DCA (Sefc *et al.*, 2000) in na osnovi rezultatov genotipizacije je bila preučena genetska podobnost 19-ih oljčnih sort. Rezultati razvrščanja v skupine so pokazali, da je 'Črnica', ki je bila v preteklosti najbolj razširjena sorta v Slovenski Istri, genetsko podobna toskanski skupini sort, kar potrjuje domneve Huguesa (1999). Večja genetska podobnost sort je bila opažena pri sortah z večjimi plodovi. V to skupino so se uvrstile tudi nekatere lokalne istrske sorte z večjimi plodovi. Razen toskanske skupine sort pa ni bilo opaziti večje genetske povezanosti sort glede na geografski izvor.

Genetsko variabilnost 130 vzorcev oljk, ki pripadajo skupini 67 sort, so z mikrosateliti serije DCA preučili Lopes *et al.* (2004). Ugotovili so, da obstaja velika variabilnost znotraj sort, saj so pri nekaterih oljkah odkrili razliko v več kot 15% namnoženih alelov. Z genotipizacijo pri nekaterih sortah niso potrdili sinonimov, omenjenih v podatkovni bazi FAO. Pri več kot 60% vzorcev pa so ugotovili homonime ali napačno označitev vzorcev v kolekciji. Mikrosateliti so se v študiji pokazali kot primerno orodje pri upravljanju kolekcij, identifikaciji sort in klonov ter reševanju sinonimov in homonimov.

Montemurro *et al.* (2005) so preučili genetsko podobnost 111-ih akcesij oljk (60 sort) iz Italije, Španije, Francije in Grčije. Polimorfizem in genetsko podobnost so analizirali s tremi kombinacijami AFLP markerjev in z 27-imi mikrosatelitskimi lokusi. S kombinacijo obeh markerskih sistemov so ločili vse genotipe oljk. V dendrogramu so bile sorte razvrščene v tri večje skupine glede na uporabnost plodov: za olje, za namizne oljke in kombinacija obeh.

Mikrosateliti gojenih oljk se zaradi ohranjenosti nukleotidnih zaporedij lahko uporabljajo tudi za preučevanja genetske variabilnosti na nivoju vrst iz rodu *Olea*. Taksonomsko je oljka *(Olea europaea* L.) razdeljena v 4 podvrste, ki uspevajo v Sredozemlju, Afriki in Aziji (subsp. *europaea, cuspidata, laperrinei, cerasiformis)* (Green & Wickens, 1989), nedavno pa sta bili klasificirani še dve podvrsti *(guanchica in maroccana)* (Vergas *et al.*, 2001). Kompleks *Olea* so Rallo *et al.* (2003) preučili s 4-imi mikrosatelitskimi lokusi gojene oljke serije IAS-oli. S študijo so želeli preveriti in potrditi ohranjenost mikrosatelitskih regij pri 15-ih vrstah in podvrstah rodu *Olea*. V analizo so vključili tudi 14 oljčnih sort. Namnoževanje vseh štirih mikrosatelitskih regij so dosegli pri 13-ih taksonih, 2 vrsti pa oljčnih mikrosatelitov nista namnožili. Velik polimorfizem namnoženih markerjev je omogočal nedvoumno identifikacijo večine vzorcev, skupno so odkrili 67 alelov in od tega je bilo več kot 50% svojevrstnih. Največ polimorfnih markerjev je bilo opaženih na lokusu IAS-oli11, nekoliko manj pa na lokusih IAS-oli17 in IAS-oli22. Ti rezultati potrjujejo uporabnost lokusov serije IAS-oli za preučevanje filogenije oljke, zanimivo pa je tudi dejstvo, da so Rallo *et al.* (2000) pri preučevanju 46-ih sort z isto serijo mikrosatelitov ugotovili bistveno manj alelov (26). Rezultati razvrščanja vzorcev v sorodnostne skupine so pokazali, da imajo lokusi sorodnih vrst verjetno različno genetsko ozadje, zaradi katerega je težko določiti dejansko genetsko sorodnost med preučevanimi vrstami. Le z lokusom IAS-oli12 so dosegli logično razmestitev taksonov v podobnostne skupine, zato poudarjajo, da je za uspešnost ugotavljanja genetske podobnosti med vrstami in podvrstami oljke izbira ustreznega lokusa ključnega pomena. Znotraj vrste je dolžinski polimorfizem primerno merilo, pri večji oddaljenosti taksonov pa je treba predhodno preučiti mutacijski mehanizem in evolucijo mikrosatelita, sicer lahko izbira neustreznega lokusa privede do napačne interpretacije rezultatov. Kljub temu da so v študiji uporabili le 4 mikrosatelitske lokuse, je razvrstitev v sorodnostne skupine pokazala jasno ločitev med podvrstami in sortami. Na osnovi ugotovljenega polimorfizma je bila potrjena uporabnost lokusov za preučevanje genetske podobnosti v kompleksu *Olea*.

Genetsko raznolikost *Olea europaea* subsp. *laperrinei* sta z mikrosateliti preučila Baali-Cherif & Besnard (2005). Subsp. *laperrinei* je prvotno uspevala v saharskem gorovju, kasneje pa se je razširila še v druga afriška območja. Predvidevajo, da se je zaradi prilagajanj različnim in ekstremnim ekološkim razmeram izoblikovalo več ločenih populacij. Avtorja sta v raziskavo vključila 111 dreves podvrste *laperrinei* in 34 dreves podvrste *europaea* iz Alžirije. Na osnovi objavljenih karakteristik mikrosatelitov sta za analizo variabilnosti izbrala 8 lokusov gojene oljke (DCA1, DCA3, DCA8, DCA9, DCA14, DCA15, GAPU 45, EMO3) in lokus $PA(ATT)_2$ bližnjega sorodnika oljke (*Phillyrea angustifolia* L.). Lokusi so bili izbrani na osnovi števila namnoženih alelov in njihovih dolžin, da so lahko opravili namnoževanje več lokusov hkrati. Z namenom, da bi se izognili pojavu ničtih alelov, sta upoštevala tudi vrednosti opažene in pričakovane heterozigotnosti posameznih lokusov. Število namnoženih alelov je bilo pri obeh podvrstah *europaea* (85 alelov) in *laperrinei* (89 alelov) podobno. V skupini oljčnih sort podvrste *europaea* sta največ alelov (16) odkrila na lokusih DCA8, DCA9, sledili so jima lokusi DCA14 (13 alelov), EMO3 (12

alelov) in DCA1 (10 alelov). Najmanj alelov pa je bilo ugotovljenih na lokusu GAPU45 (2). Podobne rezultate sta dobila tudi pri analizi podvrste *laperrinei*. Zanimivo je, da sta na lokusu DCA1 pri tej podskupini odkrila kar 24 različnih alelov. Na tem lokusu smo pri preučevanju 19-ih oljčnih sort iz nacionalnega kolekcijskega nasada v Sloveniji ugotovili le 5 alelov (Bandelj *et al.*, 2004b), drugi dve skupini pa le po 4 alele (Sefc *et al.*, 2000; Lopes *et al.*, 2004). Število namnoženih alelov na lokus ni torej vedno zanesljivo merilo za izbiro najboljših lokusov za genetske analize oljke in je lahko v veliki meri odvisno od raznolikosti genetskega materiala, ki je vključen v raziskavo. Na lokusu DCA1 sta avtorja ugotovila tudi trialelni profil pri treh vzorcih populacije *laperrinei*, kar pripisujeta somatskim mutacijam in ohranitvi himerizma. Raziskava populacij podvrste *laperrinei* je pokazala, da je tudi znotraj majhnih populacij možno odkriti veliko variabilnost, kljub nespolnemu načinu razmnoževanja.

Uporabnost mikrosatelitov oljke v žlahtnjiteljskih programih

Mikrosateliti se lahko rutinsko uporabljajo tudi v žlahtnjiteljskih programih oljk. De la Rosa *et al.* (2004) so mikrosatelite uporabili za testiranje starševstva potomcev štirih avtofertilnih sort in potomcev sedmih kontroliranih križanj, ki so jih opravili v Španiji v programu žlahtnjenja oljke. Za analizo so med poznanimi mikrosateliti izbrali 8 lokusov serije EMO (De la Rosa *et al.*, 2002) in DCA (Sefc *et al.*, 2000) in najprej opravili genotipizacijo 23-ih oljčnih sort. Avtorji so ocenili informacijsko vrednost lokusov na osnovi števila namnoženih alelov in sposobnosti ločevanja sort s kombinacijo alelov (genotipov) na posameznem lokusu. Največ sort so lahko ločili z lokusi EMO2 (11), EMO3 (10), DCA9 (16) in DCA18 (12), slabšo ločevalno sposobnost markerjev pa so opazili na lokusih EMO13, EMO30, EMO88 in EMO90. Lokuse z visoko informacijsko vrednostjo so nato uporabili za testiranje starševstva 149-ih potomcev. Nestarševske alele so ugotovili tako pri avtofertilnih rastlinah kot pri potomcih načrtovanih križanj. Pravilni alelni profili so bili ugotovljeni le pri treh kontroliranih križanj sort Picual in Arbequina. Z raziskavo so avtorji podali tudi nekaj pomembnih ugotovitev za načrtovanje križanj oljk. Ugotovili so, da je emaskulacija pri avtofertilnih sortah oljk nepotrebna in da je za zagotavljanje uspešnosti križanja potrebno opraševalne vrečke nameščati na materine rastline, preden se v zraku pojavi prvi pelod. Avtorji so 23 oljčnih sort na osmih lokusih predstavili z alelnimi profili v bp, kar bo v nadaljevanju omogočalo primerjavo rezultatov genotipizacije oljk med različnimi laboratoriji, kar pomeni velik prispevek k vzpostavljanju podatkovne baze za genotipizacijo oljke z mikrosatelitskimi markerji.

Preverjanje pristnosti sortne strukture oljčnih olj z oznako zaščiteno geografsko poreklo

Z Uredbo o zaščiti geografskih označb in označb porekla za kmetijske proizvode in živila (ES št. 510/2006) se oljčna olja z geografskim poreklom pridobivajo iz določenega sortimenta, ki je za območje značilen. Tehnologija DNA ponuja obetaven način kontrole provenience oljčnega olja, saj je v vsakem olju prisotna DNA sort oljk, iz katerih je bilo olje pridobljeno (Breton *et al.*, 2004; Woolfe & Primrose, 2004). V Italiji (Muzzalupo & Perri, 2002; Busconi *et al.*, 2003; Testolin & Lain, 2005; Pafundo *et al.*, 2005) in Franciji (Breton *et al.*, 2004) že poročajo o uspešni vzpostavitvi kontrole sledljivosti oljčnih olj z markerji DNA in aplikacije metode za preverjanje geografskega porekla. Raziskovalci pri tem poudarjajo, da je ključnega pomena ustrezna izbira molekulskih markerjev ter predhodno vzpostavljena podatkovna baza z opisi markerjev za sorte nekega pridelovalnega območja (Busconi *et al.*, 2003; Pasqalone *et al.*, 2004). Prve raziskave o uporabi mikrosatelitov pri določanju sort v oljčnem olju so pokazale, da so ti markerji primerni in najbolj perspektivni za genetsko kontrolo pristnosti oljčnih olj z geografskim poreklom (Breton *et al.*, 2004; Pasqualone *et al.*, 2004, Testolin & Lain, 2005).

Uporabnost mikrosatelitov pri kartiranju genoma oljke

Motivi za izdelavo genskih kart so lahko različni. Pri rastlinah se uporabljajo za kartiranje lokusov, povezanih s kvalitativnimi (enostavnimi) ali kvantitativnimi lastnostmi (QTL: Quantitative Trait Locus). Izdelana genska karta lahko rabi kot orodje, ki ga potrebujejo žlahtnjitelji pri načrtovanju procesa izboljšanja genskih virov rastlin. Gensko kartiranje temelji na principu, da se geni, ki so dovolj blizu na istem kromosomu, dedujejo vezano. Pri tem se ugotavlja pogostost rekombinacij, pri čemer se lahko oceni razdalja med geni ali genskimi markerji na istem kromosomu. Mikrosateliti so zaradi številčnosti v rastlinskih genomih zelo uporabni pri kartiranju.

Prvo vezano karto oljke so izdelali De la Rosa *et al.* (2003), v katero so vključili markerje RAPD, AFLP, RFLP in mikrosatelite. Analizo dedovanja so opravili na 95-ih potomcih psevdotestnega križanja dveh visoko heterozigotnih sort 'Leccino' in 'Dolce Agogia'. Pripravljeni karti sta povezali 249 markerjev s pokritostjo 2765 cM pri sorti 'Leccino' in 236 markerjev s pokritostjo 2445 cM pri sorti 'Dolce Agogia'. Zaradi prisotnosti manjših vezanih skupin in nevezanih markerjev se karti nista razdelili v 23 vezanih skupin, kot so pričakovali. Avtorji sklepajo, da uporabljeni markerji niso enakomerno razpršeni v genomu oljke.

Wu *et al.* (2004) so predstavili prvo integrirano karto oljke, ki so jo pripravili po principu psevdotestnega križanja sort 'Frantoio' in 'Kalamata'. V analizo so vklju-

čili 104 potomce F_1 generacije in preučili dedovanje 178 markerjev RAPD, 9 mikrosatelitov in 3 markerje SCAR, med katerimi je bil en povezan z odpornostjo na glivo pavjega očesa. Integrirana karta je vsebovala 101 lokus, ki so bili razdeljeni v 15 skupin s povprečno razdaljo med lokusi 10,2 cM. Po prehodu potomcev križanja v rodno obdobje nameravajo avtorji v obstoječo gensko karto vključiti še morfološke markerje agronomsko pomembnih lastnosti, kot so čas cvetenja, odpornost na bolezni ter kakovostne parametre oljčnega olja in plodov (npr. oljevitost). Vezana karta bo temelj za bodoče programe žlahtnjenja oljk in bo uporabljena za identifikacijo lokusov, ki določajo kvantitativne lastnosti (QTL), ter za preučevanje genoma oljke.

ZAKLJUČKI

Lastnosti in prednosti mikrosatelitskega markerskega sistema pred drugimi razpoložljivimi markerji so dobro poznane. Z razvojem mikrosatelitov oljke so se ti markerji vključili v različne genetske študije: genotipizacija in identifikacija oljčnih sort, ugotavljanje genetske variabilnosti klonov in sort, preučevanje genetske podobnosti in strukture oljk različnih geografskih območji, izdelava genskih kart in starševske analize. Mikrosateliti veliko obetajo tudi na področju kontrole sortne sestave oljčnega olja z zaščitenim geografskim poreklom. V identifikacijskih študijah se je pokazalo, da bi bilo treba pri oljki vzpostaviti poenoten postopek identifikacije genotipov in podatkovno bazo z opisi referenčnih sort na nivoju DNA. Izbira mikrosatelitov se zdi logična, saj bo s tem omogočena primerljivost rezultatov med laboratoriji. Podatkovna baza z opisi oljčnih sort na visoko informativnih lokusih bo prispevala k pospešeni identifikaciji in karakterizaciji genskih virov oljke, rešitvi sinonimov in homonimov ter ugotavljanju geografskega izvora oljk. Vzpostavitev podatkovne baze genotipov oljk pridelovalnih območij je nujno potrebna tudi z vidika zagotavljanja genetske kontrole oljčnih olj s kakovostnimi oznakami.

Uporaba mikrosatelitskega markerskega sistema v genetskih študijah vključuje tudi analize informativnosti lokusov, saj vsi objavljeni mikrosateliti niso primerni kot markerji v molekularno-genetskih študijah. Razlikujejo se v kakovosti namnoževanja alelov in sposobnosti odkrivanja polimorfizma. Iz tega vidika je pomembno objavljene markerje predhodno testirati in izbrati le najbolj informativne glede na cilj raziskave. Najpogostejši kriteriji pri izbiri lokusov so število namnoženih alelov, heterozigotnost lokusa, informacijska vrednost polimorfizma (PIC vrednost) in verjetnost identičnosti genotipov (PI vrednost). Izbira je lahko tudi kompleksnejša, če upoštevamo, da so nekatere karakteristike markerjev odvisne tudi od strukture mikrosatelita. Pojavi ničtih alelov in senčnih fragmentov ter večlokusno namnoževanje mikrosatelitov lahko privedejo do napačne interpretacije rezultatov, zato je pri izbiri najprimernejših lokusov za analize treba upoštevati tudi te kriterije.

Med objavljenimi mikrosateliti oljke so kar 4 študije potrdile izredno visoko informacijsko vrednost lokusov serije ssrOeUA-DCA (Sefc *et al.*, 2000; Bandelj *et al.*, 2004b; Lopes *et al.*, 2004; La Mantia *et al.*, 2005). Če povzamemo, so bili lokusi DCA3, DCA9, DCA10 in DCA16 najprimernejši za identifikacijo oljčnih sort zaradi nizke verjetnosti identičnosti genotipov. Zavoljo nizke informacijske vrednosti in visoke verjetnosti identičnosti genotipov ter majhnega števila efektivnih alelov so bili najslabši parametri variabilnosti opaženi pri lokusih DCA1, DCA5, DCA13 in DCA15. Verjetnost pojava ničtih alelov je bila največja pri lokusih DCA4, DCA11 in DCA13. Lokusa DCA4 in DCA14 namnožujeta kompleksne alelne profile, pri lokusih DCA1, DCA10 in DCA17 je bila ugotovljena dominanca kratkega alela (Bandelj *et al.*, 2004b), lokusa DCA11 in DCA17 pa imata nestabilno mikrosatelitsko strukturo (Sefc *et al.*, 2000).

V seriji mikrosatelitov IAS-oli (Rallo *et al.*, 2000) so bili ugotovljeni najboljši parametri variabilnosti pri dveh lokusih IAS-oli11 in IAS-oli12, slabši pa pri lokusih IAS-oli06, IAS-oli17 in IAS-oli22. Rezultati dveh študij (Rallo *et al.*, 2000, 2003) so pokazali, da so lokusi serije IAS-oli morda primernejši za raziskave kompleksa *Olea*, saj je bilo pri preučevanju različnih podvrst oljke ugotovljeno bistveno večje število markerjev (67) v primerjavi s študijo 46-ih sort, kjer so odkrili le 26 mikrosatelitov.

Trideset objavljenih mikrosatelitskih lokusov serije UDO (Cipriani *et al.*, 2002) je imelo v povprečju le po 3 mikrosatelite na lokus. Vzrok za tako nizko število ugotovljenih alelov je verjetno v ožji genetski podobnosti 12-ih sort, ki so jih avtorji vključili v analizo. La Mantia *et al.* (2005), ki so uporabili 6 UDO lokusov v študiji genetske strukture 46-ih akcesij oljk, so v povprečju namnožili bistveno več mikrosatelitov (7,8). Slabše parametre variabilnosti so odkrili na lokusu UDO-009, opažena heterozigotnost pa je bila nižja od pričakovane na lokusih UDO-008 in UDO-039, zato ti lokusi niso najbolj primerni za genetske študije.

Sodeč po številu namnoženih alelov na lokusih serije GAPU (Carriero *et al.*, 2002) bi lahko med 20 objavljenimi lokusi izbrali kot primerne za genetske študije lokuse: GAPU101, GAPU103A, GAPU47, GAPU89, GAPU71B, GAPU71A, GAPU45 in GAPU59. La Mantia *et al.* (2005) so potrdili visoko informacijsko vrednost dveh lokusov: GAPU101 in GAPU59.

Dobri parametri variabilnosti so bili ugotovljeni tudi za serijo mikrosatelitskih lokusov EMO (De la Rosa *et al.*, 2002). Na vseh lokusih razen EMOL so odkrili več kot 6 alelov, za EMO30 je bil značilno večlokusno namnoževanje markerjev, pri EMO90 pa je bila opažena heterozigotnost nižja od pričakovane. Nekoliko slabša sposobnost ločevanja sort je bila ugotovljena pri lokusih EMO13 in EMO88. Med informativne lokuse bi torej lahko uvrstili lokusa EMO2 in EMO3.

MICROSATELLITE MARKERS AND THEIR USE IN OLIVE GROWING

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SUMMARY

Microsatellites as one of the most popular marker systems are widely used in plant genetic research for diversity studies, linkage analysis, genetic map development and fingerprinting studies. Their wide usage is based on their excellent properties, such as high abundance in eucaryotic genomes, co-dominant nature, hypervariability, robustness and high information content. In olives, first microsatellites were published in 2000, and from then on they have been frequently included in different olive genetic investigations. The objective of this work was to present published microsatellites in olives, to review their characteristics and to survey their applicability in olive genetic studies.

Key words: microsatellites, SSR, *Olea europaea* L., olive growing, polymorphism

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SPREMLJANJE POJAVA OLJČNE MUHE *(BACTROCERA OLEAE)* V SLOVENSKI ISTRI V LETU 2005 Z NOVO METODO ZA FITOSANITARNO VARSTVO OLJK

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IZVLEČEK

Oljčna muha (Bactrocera oleae) *je gospodarsko najpomembnejši škodljivec oljk v sredozemskih državah. Z namenom, da bi zmanjšali gospodarsko škodo, ki jo oljčna muha povzroča v Slovenski Istri, smo v okviru projekta SIGMA (Program pobude skupnosti INTERREG IIIA Slovenija-Italija) uvedli mrežo za nadzor oljčne muhe. Let oljčne muhe smo spremljali z uporabo feromonskih vab, ki smo jih namestili na 30 vzorčnih mest. Vabe smo tedensko pregledovali v obdobju od 13.7.2005 do 15.10.2005. Poleg spremljanja leta oljčne muhe smo na vzorčnih mestih spremljali tudi okuženost oljčnih plodov. Rezultati so pokazali, da se zaradi geografske in mikroklimatske heterogenosti Slovenske Istre pojavlja različna intenzivnost napada oljčne muhe. Največje število ulovljenih oljčnih muh je bilo zabeleženo v tednu med 11.9. in 17.9.2005. Največja skupna okuženost plodov (8,46%) je bila ugotovljena v tednu tik pred obiranjem pridelka (30.10.–5.11.2005). Aktivna okuženost (5,11%) je v tem obdobju presegla prag škodljivosti (5,0%). Vzpostavitev mreže za nadzor oljčne muhe je finančno podprla Evropska unija.*

Ključne besede: oljčna muha, Slovenska Istra, fitosanitarna mreža spremljanja

MONITORAGGIO DELLA MOSCA DELL'OLIVO *(BACTROCERA OLEAE)* NELL'ISTRIA SLOVENA NEL 2005 CON LA NUOVA METODOLOGIA FITOSANITARIA PER LA TUTELA DEGLI OLIVI

SINTESI

La mosca dell'olivo (Bactrocera oleae) *è il parassita piu' nocivo all'olivicoltura nei paesi europei. Al fine di diminuire i danni economici provocati dalla mosca dell'olivo nell'Istria slovena, gli autori hanno introdotto una rete di monitoraggio del parassita, nell'ambito del progetto SIGMA (programma incentivato da INTERREG IIIA Slovenia-Italia). Il volo della mosca dell'olivo è stato monitorato con l'aiuto di esche di feromoni, posizionate in 30 stazioni di campionamento. Le esche venivano controllate settimanalmente, nel periodo dal 13.7.2005 al 15.10.2005. Nelle stazioni di campionamento veniva controllato anche il contagio dei frutti. I risultati indicano che a causa dell'eterogeneità geografica e microclimatica dell'Istria slovena si verificano diverse intensità di attacco della mosca dell'olivo. Il maggior numero di mosche dell'olivo è stato catturato nella settimana fra l'11.9. ed il 17.9.2005. Il contagio maggiore di frutti (8,46%) è stato riscontrato nella settimana antecedente il raccolto (30.10.-5.11.2005). Il contagio attivo (5,11%) in tale periodo ha superato la soglia di nocività (5,0%). L'introduzione della rete di monitoraggio della mosca dell'olivo ha avuto l'appoggio finanziario dell'Unione Europea.*

Parole chiave: mosca dell'olivo, Istria slovena, rete fitosanitaria di monitoraggio

UVOD

Na sredozemskem območju ima gojenje oljk velik socialno-ekonomski pomen, saj pokriva kar 98% svetovne pridelave oljk. Oljčni nasadi zavzemajo skupno 10.000.000 ha površin (800.000.000 dreves oljk), na katerih se letno pridela od 2,3 do 3,2 milijona ton oljčnega olja in od 1,2 do 1,7 milijona ton vloženih oljk (http://www.internationaloliveoil.org/downloads/market-august06.pdf).

Količina pridelka oljk se lahko zaradi pojava škodljivcev, glivičnih bolezni in plevelov zmanjša za več kot 30%. Pridelovalci oljk samo za zatiranje škodljivcev letno namenijo 100.000.000 dolarjev, vendar se kljub temu ob napadu škodljivcev pridelek zmanjša za 15%, kar pomeni 800.000.000 dolarjev škode (Bueno & Jones, 2002).

Med gospodarsko najpomembnejše škodljivce oljk uvrščamo oljčno muho (*Bactrocera (Dacus) oleae* (Gmelin, 1788)), ki ima na območju Sredozemlja letno od dva do pet rodov (Mazomenos *et al.*, 2002). Pojav oljčne muhe je v veliki meri odvisen od klimatskih razmer, saj se pri visoki zračni vlagi in temperaturi med 20 in 30 °C število oljčnih muh znatno poveča. Če se temperatura zraka zviša nad 30 °C, relativna vlažnost pa zniža, se plodovi oljke posušijo. Ličinke oljčne muhe v takšnih razmerah ne preživijo (Civantos, 1999). Samica oljčne muhe odloži po eno jajčece v plod oljke, ko le ta doseže določeno velikost in koščica v njem otrdi (Phillips & Rice, 2001). V življenjskem ciklusu odloži od 200 do 500 jajčec (Zalom *et al.*, 2003), iz katerih se izležejo ličinke, ki se hranijo z vrtanjem mesnatega dela plodov, dokler ne predrejo epiderme in se prelevijo v pupe. Po številnih metamorfozah ličinke iz ploda odleti odrasla muha (Civantos, 1999). V napadenih plodovih se pričnejo oksidacijski procesi, ki vplivajo na povečanje prostih maščobnih kislin, zaradi česar se v oljih poveča kislinsko število, kar močno poslabša njegovo kakovost. Določitev prostih maščobnih kislin je osnovni parameter pri določanju kakovosti oljčnega olja (Bučar Miklavčič, 1998).

Oljčno muho zatiramo z različnimi biotehniškimi ukrepi: 1) klasično ali kurativno metodo uporabimo, ko so plodovi že napadeni, 2) z integrirano ali preventivno metodo preprečimo odlaganje jajčec v plodove ter 3) ekološko metodo, pri kateri je možna uporaba tako proteinskih zastrupljenih vab kakor tudi vab za množičen ulov. Poudariti velja, da imajo na oljčno muho odvračalni in razkuževalni učinek tudi bakrovi pripravki, ki sicer delujejo proti boleznim oljk (Jančar *et al.*, 2005).

Kurativno metodo varstva uporabimo, ko ima več kot 10% plodov fertilni vbod. V tem primeru z dimetoatom (Perfekthion) ali triklorfonom (Dipterex 80%) škropimo po celi krošnji (Tehnološka navodila za integrirano pridelavo sadja, 2006).

Pri integriranemu varstvu je prag škodljivosti dosežen, ko se na rumeno lepljivo ploščo ulovijo tri oljčne muhe na teden oz. se pri 5% plodov pojavi fertilni vbod oljčne muhe. Integrirana pridelava oljk temelji na uporabi hidroliziranih proteinskih vab (Nulure) z dodatkom dimetoata (Perfekthion). Pri preventivnem škropljenju oljk proti oljčni muhi poškropimo le manjši, nerodni del krošnje in debla na južni strani dreves. Takšen način aplikacije lahko opravimo največ 5-krat na leto (Tehnološka navodila za integrirano pridelavo sadja, 2006).

Najbolj ekološko neoporečen in okolju prijazen biotehniški ukrep za varstvo oljk je biološka metoda, saj zatiranje oljčne muhe ne temelji na uporabi kemičnih sredstev, ampak na uporabi raztopine ocetne kisline.

Analiza plodov, kjer ugotavljamo odstotek plodov s fertilnimi vbodi (aktivna in škodljiva okuženost plodov) in tedensko pregledovanje naleta oljčnih muh na rumenih lepljivih ploščah, sta enostavni in hitri metodi za določanje praga škodljive okuženosti oljčnih plodov in praga pojava oljčne muhe, vendar nam ne zagotavljata podatka o dejanski škodi, ki jo oljčna muha povzroča na plodovih oljke. Z uvedbo nove metode za fitosanitarno varstvo oljk v okviru čezmejnega projekta SIGMA "Inovativni sistem za skupno upravljanje v kmetijskem sektorju in skupna uporaba čezmejne mreže za kmetijsko okoljsko monitoriranje" poskušamo posredno vplivati na izboljšanje kakovosti oljčnega olja in na zmanjšanje gospodarske škode, ki jo oljčna muha povzroča v Slovenski Istri. Z rednim spremljanjem oljčne muhe v oljčnikih in kontroliranim ukrepanjem bo zagotovljena tudi manjša obremenitev okolja Slovenske Istre.

MATERIAL IN METODE

Na območju Slovenske Istre smo v začetku julija 2005 vzpostavili mrežo za nadzor oljčne muhe, v katero je bilo vključenih 30 vzorčnih mest. Referenčne lokacije so bile izbrane na osnovi geografske lege oljčnikov (obalni pas in zaledje) ter glede na način varstva oljk pri posameznem pridelovalcu (Tab. 1). Na podlagi načina varstva oljke smo izbrali 24 vzorčnih mest, kjer pridelovalci tretirajo po priporočilih integrirane metode 3 vzorčna mesta s klasično metodo ter 3 vzorčna mesta, kjer se opravlja varstvo po načinu biološke metode.

Tab. 1: Vzorčne lokacije in načini fitosanitarnega varstva oljk.
Tab. 1: Sampling sites and modes of phytosanitary olive tree protection.

Območje Slovenske Istre / The area of Slovene Istra	**Vzorčno mesto – pridelovalec / Sampling site – grower**	**Metoda varstva rastlin / Olive tree protection method**
Obalni pas Slovenske Istre / Coastal part of Istra	Baredi – Dušan Moljk	Integrirano
	Beneša 1 – Jevnikar Aleksander	Integrirano
	Beneša 2 – Dino Pucer	Integrirano
	Ronek – Viljanka Vesel	Integrirano
	Sermin – Miran Sotlar	Integrirano
	Seča – Aleksander Grbec	Integrirano
	Plavje 1 – Erika Čok	Integrirano
	Plavje 2 – Neva Šečerov	Integrirano
	Liminjan – Vlado Munda	Integrirano
	Makovec – Franko Miklavčič	Integrirano
	Mala Seva – Vanja Dujc	Integrirano
	Pobegi – Ido Kocijančič	Integrirano
	Beneša 3 – Angel Pucer	Biološko
	Strunjan – Sonja Mikulin	Biološko
Zaledje Slovenske Istre / Hinterland of Slovene Istra	Gažon – Matjaž Jančar	Integrirano
	Potok – Darko Jakomin	Integrirano
	Lama 1 – Angelo Hlaj	Integrirano
	Lama 2 – Oskar Furlanič	Integrirano
	Šmajre –Albin Gec	Integrirano
	Bonini – David Olivo	Integrirano
	Osp 1 – Dušan Bandelj	Integrirano
	Osp 2 – Peter Vovk	Integrirano
	Grbci – Beno Bajda	Integrirano
	Korte – Zdenko Hrvatin	Integrirano
	Padna – Aldo Pucer	Integrirano
	Krkavče – Robert Lisjak	Integrirano
	Triban – Boris Gorup	Klasično
	Sv. Peter – Emil Feran	Klasično
	Truške – Janko Bočaj	Klasično
	Sv. Peter – Janez Forte	Biološko

Na vsakem vzorčnem mestu smo na južno stran krošnje izbranega oljčnega drevesa namestili rumeno lepljivo ploščo s feromonsko vabo (Dacotrap-ISAGRO-Italija), ki vsebuje naravne izločke spolno zrelih samičk in tako privablja spolno zrele samčke oljčne muhe. Od 13.7.2005 dalje smo spremljali tedensko dinamiko leta oljčne muhe in beležili število ujetih samčkov in samičk na vabah. Na podlagi upoštevanja 21- dnevne karence, ki mora preteči od zadnjega tretiranja s fitofarmacevtskim sredstvom do obiranja oljk, smo 15.10.2005 spremljanje zaključili. Pridobljene podatke smo analizirali z metodo, ki so jo za analizo leta oljčne muhe razvili v letih 1999–2001 v okviru projekta Miglioramento della qualita' dell'olio di oliva na Visoki šoli Svete Ane v Pisi (Italija) (Petacchi *et al.*, 2001a).

V obdobju 12 tednov (od 13.7. do 15.10.2005) spremljanja leta oljčne muhe je potekalo tudi vzorčenje oljčnih plodov. Na vsaki izbrani lokaciji smo tedensko naključno nabrali 100 plodov, ki so bili takoj po vzorčenju pregledani pod stereomikroskopom Motic. Tako smo lahko določili razvojni stadij oljčne muhe (jajčece, ličnika 1. stadija, ličinka 2. stadija, ličinka 3. stadija, buba, imago), na podlagi katerega smo ugotovili odstotek aktivne, škodljive in skupne okuženosti plodov.

Če se v plodu oljke pojavijo jajčeca, žive ličinke 1. stadija ali žive ličinke 2. stadija, so plodovi aktivno okuženi. Škodljiva okuženost pa se pojavi, ko v plodu oljke zabeležimo žive ličinke 3. stadija, bube ali odrasle muhe oz. izhodno odprtino, skozi katero je muha odletela. Skupna okuženost je tako vsota škodljive in aktivne okuženosti plodov (Petacchi *et al.*, 2001b).

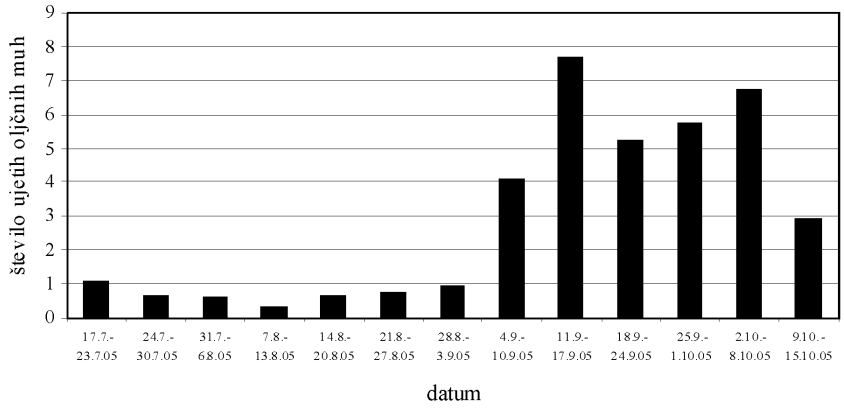
Oljčna muha poleti odloži jajčeca v plodove s trdo koščico, jeseni pa za odlaganje jajčec izbere manj zrele plodove, zato smo tedensko s pomočjo BBCH-identifikacijskega ključa (Sanz-Cortes *et al.*, 2002) določali tudi fenološki stadij plodov.

REZULTATI IN RAZPRAVA

Dinamika pojavljanja oljčne muhe v slovenskih oljčnikih se v okviru strokovnega dela kmetijske svetovalne službe spremlja že od leta 1983 (Jančar, osebna informacija). V letu 2005 smo v Slovenski Istri pristopili k novemu načinu spremljanja oljčne muhe, ki je bil prvotno vzpostavljen v Liguriji (Italija) (1999–2001) in Toskani (Italija) v letih 2000–2001. Novejši način spremljanja gospodarsko najpomembnejšega škodljivca oljk vključuje poleg spremljanja leta oljčne muhe na feromonskih vabah še redno pregledovanje oljčnih plodov, s čimer ugotavljamo razvojno fazo škodljivca in dejansko škodljivost, ki jo pri tem povzroča. Glavni cilj vzpostavitve mreže spremljanja oljčne muhe je zmanjšati število fitosanitarnih ukrepov v oljčnikih in tako zmanjšati obremenitve okolja. Posredno bo kontrolirano tretiranje s fitofarmacevtskimi sredstvi vplivalo še na izboljšano kakovost oljčnega olja.

V obdobju med 17.7. in 28.8.2005 smo na vseh opazovanih lokacijah Slovenske Istre zabeležili nizko število ulovljenih oljčnih muh. Povečan let oljčne muhe smo zasledili v 36. tednu (4.–10.9.2005), ko je bilo zabeleženih v povprečju 4,12 oljčne muhe na feromonsko vabo (Tab. 2). Največ ulovljenih oljčnih muh smo zabeležili v 37. tednu (11.–17.9.2005).

Najbolj izpostavljeni lokaciji za napad oljčne muhe v Slovenski Istri sta Strunjan in Ronek, saj smo v obdobju od 15.7. do 16.10.2005 na teh opazovanih mestih zabeležili največje število ulovljenih oljčnih muh, medtem ko smo na vzorčnih mestih Truške in Baredi v celotnem opazovanem obdobju skupno zabeležili le 5 ulovljenih oljčnih muh. Iz teh rezultatov sklepamo, da so razmere v oljčnikih, ki so locirani v bližini morja, zaradi domnevno višje relativne vlage ugodnejše za pojav oljčne muhe. Znano je, da nizke doline z visoko vlažnostjo zraka, bližina morja ter zmerno topla deževna poletja ustvarijo ugodne razmere za razvoj oljčne muhe, kar znatno vpliva na velikost populacije (Cirio, 1998). Kljub temu da so nameščene feromonske vabe vsebovale naravne izloč-



Sl. 1: Dinamika leta oljčne muhe** (Bactrocera oleae) **v Slovenski Istri v letu 2005.
Fig. 1: Flight dynamics of olive fly** (Bactrocera oleae) **in Slovene Istra during 2005.

Tab. 2: Ulov oljčne muhe v obdobju od 15.7. do 16.10.2005 v Slovenski Istri.
Tab. 2: Olive fruit flies caught between 15 Jul and 16 Oct 2005 in Slovene Istra.

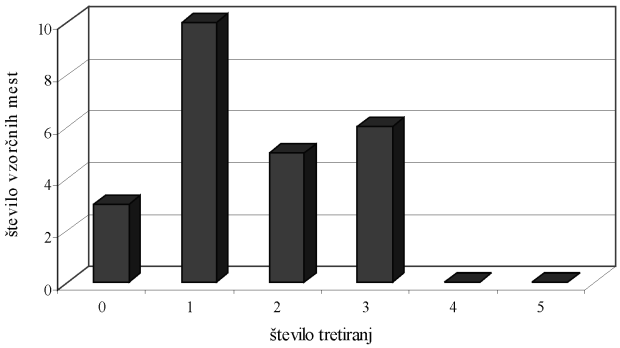
Teden v letu / Week in the year	Datum / Date	Število vzorčnih mest / No. sampling posts	Povprečje / Mean	Max	Min
29.	17.7.–23.7.2005	21	1,05	5,00	0,00
30.	24.7.–30.7.2005	22	0,64	2,00	0,00
31.	31.7.–6.8.2005	23	0,57	2,00	0,00
32.	7.8.–13.8.2005	21	0,29	2,00	0,00
33.	14.8.–20.8.2005	21	0,62	3,00	0,00
34.	21.8.–27.8.2005	20	0,75	3,00	0,00
35.	28.8.–3.9.2005	18	0,94	4,00	0,00
36.	4.9.–10.9.2005	14	4,12	14,00	0,00
37.	11.9.–17.9.2005	11	7,64	24,00	1,00
38.	18.9.–24.9.2005	11	5,18	14,00	0,00
39.	25.9.–1.10.2005	11	5,73	18,00	0,00
40.	2.10.–8.10.2005	10	6,70	22,00	0,00
41.	9.10.–15.10.2005	10	2,90	6,00	0,00

ke samic, ki privabljajo samo spolno zrele samce oljčne muhe, smo na rumenih lepljivih ploščah opazili tudi manjše število samic. Na sliki 1 je prikazana dinamika leta oljčne muhe v Slovenski Istri.

Na podlagi števila ujetih oljčnih muh na feromonskih vabah in okuženosti plodov so pridelovalci na vzorčnih mestih Obale z zastrupljenimi proteinskimi vabami ukrepali 2- do 3-krat, in sicer 25.7., 31.8. in 13.9.2005. V zaledju Slovenske Istre pa so ukrep proti oljčni muhi izvedli samo enkrat oziroma dvakrat (12.9. in 24.9. 2005), če so padavine škropilno brozgo izprale. Na treh vzorčnih mestih sta bila število ujetih oljčnih muh in okuženost plodov tako nizka, da tretiranje ni bilo potrebno (Sl. 2).

Pregled oljčnih plodov je zelo pomemben kazalec dejanske škode, ki jo oljčna muha povzroča. Pri aktivni okužbi se količina mesnatega dela plodov zmanjša od 3 do 10%. S pravočasnim ukrepanjem lahko s kurativnimi metodami prekinemo nadaljnji razvoj ličink 1. in 2. stadija ter jajčec, in tako preprečimo nastanek škodljive okuženosti. V primeru škodljive okuženosti, ko v plodu oljke zasledimo živo ličinko 3. stadija, bubo ali imago (izhodno odprtino), je škoda že tako velika, da jo kljub tretiranju ni mogoče več zmanjšati. Posebno pozornost je treba posvetiti tudi številu mrtvih ličink, saj tako ugotovimo, ali je bil ukrep proti oljčni muhi učinkovit. Umrljivost ličink 1. in 2. stadija se lahko poveča tudi zaradi povišane temperature v poletnem času, umrljivost ličink 3. stadija pa zaradi naravnih sovražnikov *(Eupelmus urozonus, Pnigalio mediterraneus, Eurytoma martelli)* (Delrio, 1995).

V opazovalnem obdobju smo dne 20.7.2005 zabeležili prvo odloženo jajčece v plodu oljke. V tem času je



Sl. 2: Število tretiranj proti oljčni muhi z zastrupljeno proteinsko vabo v letu 2005.
Fig. 2: No. of treatments against olive fly with poisoned protein bait in 2005.

bila skupna okuženost plodov (0,97%) nižja od praga škodljivosti (Tab. 3). Vrednost zabeležene skupne okuženosti plodov je bila do 37. tedna enaka aktivni okuženosti, saj se je škodljiva okuženost prvič pojavila po večjem letu oljčne muhe, v času od 11.–17.9.2005 (Sl. 3). Iz podatkov škodljive okuženosti lahko sklepamo, da je bila v letu 2005 dejanska škoda, ki jo je oljčna muha povzročila na plodovih oljke, nizka, saj so se vrednosti le-te gibale od 0,04 do 3,34%. Nekoliko večje vrednosti smo zabeležili za aktivno okuženost (0,97–5,11). Aktivna okuženost (5,11%) je tako v obdobju od 30.10.2005 do 5.11.2005 presegla prag škodljivosti (5,0%), ki ga predpisujejo navodila za integrirano pridelavo sadja. Prag škodljivosti kurativne metode (10%) v sezoni 2005 ni bil presežen.

Tab. 3: Skupna okuženost plodov (aktivna + škodljiva okuženost) v obdobju od 15.7. do 16.10.2005 v nasadih integrirane pridelave v Slovenski Istri.
Tab. 3: Total fruit infestation (active + detrimental) during the period from 15 Jul to 16 Oct 2005 in plantations of integrated growing in Slovene Istra.

Teden v letu / Week in the year	Datum / Date	Število vzorčnih mest / No. sampling posts	Povprečje / Mean	Max	Min
29.	24.7.–30.7.2005	22	0,97	4,00	0,00
30.	31.7.–6.8.2005	24	0,13	1,00	0,00
31.	7.8.–13.8.2005	24	0,21	1,00	0,00
32.	14.8.–20.8.2005	24	0,92	3,00	0,00
33.	21.8.–27.8.2005	24	0,50	3,00	0,00
34.	28.8.–3.9.2005	24	0,67	3,00	0,00
35.	4.9.–10.9.2005	24	1,29	8,00	0,00
36.	11.9.–17.9.2005	24	2,00	10,00	0,00
37.	18.9.–24.9.2005	24	1,31	10,00	0,00
38.	25.9.–1.10.2005	24	3,03	24,07	0,00
39.	2.10.–8.10.2005	24	3,13	18,10	0,00
40.	9.10.–15.10.2005	24	3,48	30,00	0,00
41.	30.10.–5.11.2005	24	8,46	37,86	0,00

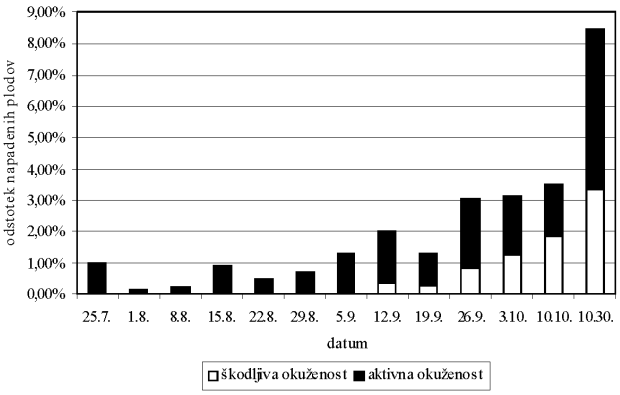
Največjo skupno okuženost plodov (8,46%) smo zabeležili v zadnjem tednu karence, pred obiranjem pridelka (30.10.–5.11.2005). Aktivna okuženost (5,11%) je v tem obdobju presegla tudi prag škodljivosti (5%), ki je predpisan v tehnoloških navodilih za integrirano pridelavo sadja. Prag škodljivosti kurativne metode (10%) v sezoni 2005 ni bil presežen.

ZAKLJUČEK

Z rednim spremljanjem leta oljčne muhe v letu 2005 smo ugotovili, da se zaradi mikroklimatske heterogenosti Slovenske Istre pojavlja različna intenzivnost napada oljčne muhe. Ne glede na heterogenost prostora ima oljčna muha v Slovenski Istri tri rodove. Prvi rod smo zabeležili v 29. tednu, ko je bilo v oljčne plodove odloženo manjše število jajčec, v 37. tednu (12.–19.9. 2005), ko se je pojavil 2. rod, pa je število jajčec skokovito naraslo.

Kljub temu da smo v 37. tednu zabeležili povečano število odloženih jajčec in število ujetih muh na feromonsko vabo, skupna okuženost plodov (2,0%) ni presegla praga škodljivosti (5,0%). Prag škodljivosti je bil presežen samo v zadnjem tednu karence pred obiranjem plodov (30.10.–5.11.2005), ko ukrepanje z zastrupljenimi vabami ni bilo več mogoče. Zastrupljene proteinske vabe, katerih uporabo priporoča preventivna metoda, so se izkazale kot učinkovite za zatiranje oljčne muhe, vendar zaradi majhne populacije oljčne muhe v letu 2005, neugodnih klimatskih razmer in enoletnih podatkov tega ne moremo z gotovostjo trditi.

Ugotovili smo, da lahko s tedenskim spremljanjem leta oljčne muhe in pregledovanjem okuženosti plodov na različnih lokacijah Slovenske Istre zmanjšamo število preventivnih ukrepov z zastrupljenimi proteinskimi vabami in s tem negativni vpliv fitofarmacevtskih sredstev na okolje. Poleg tega nam podatek o okuženosti plodov omogoča spremljanje učinkovitosti tretiranja in je lahko dober kazalec dejanske škode, ki jo oljčna muha povzroča na pridelku.



Sl. 3: Dinamika okuženosti plodov v času od 13.7. do 30.10.2005 v nasadih integrirane pridelave.
Fig. 3: Fruit infestation dynamics from 13 Jul to 30 Oct 2005 in integrated growth plantations.

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MONITORING OF THE OCCURRENCE OF OLIVE FRUIT FLY *(BACTROCERA OLEAE)* IN SLOVENE ISTRA DURING 2005 WITH A NEW PHYTOSANITARY OLIVE TREE PROTECTION METHOD

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SUMMARY

The olive fruit fly (Bactrocera oleae) *is the most serious olive pest in the Mediterranean countries. To reduce economic losses caused by olive fruit fly, we had implemented monitoring network for controlling olive fruit fly in Slovene Istria area, which was carried out within the SIGMA (INTERREG IIIA Italy-Slovenia) project. To determine olive fruit fly population, pheromone traps were placed on 30 sample locations. Traps were monitored weekly from 13th July to 15th October 2005. During this time, the olive fruit infestation was also monitored. Results of the study showed that olive fruit fly occurrence depends on geographical position of olive orchards and their microclimatic conditions. The highest number of olive fruit fly captured on traps was found in the 37th week (from 11th to 17th September 2005). In the week before the olive harvesting (30 Oct–5 Nov 2005), the highest total infestation (8.46%) was noticed. Active infestation (5.11%) in that period has exceeded the threshold infestation level (5.0%). The establishment of monitoring network of olive fruit fly was financed by the European Union.*

Key words: olive fly, Slovene Istra, phytosanitary monitoring network

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OLIVE TREE – THE SOURCE OF PHARMACODYNAMICALLY ACTIVE SUBSTANCES

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ABSTRACT

Olive and its products have been recognized as an important component of a healthy diet. Increase olive oil consumption is implicated in reduction of cardiovascular diseases, rheumatoid arthritis and a variety of cancers. In the prevention of these diseases, antioxidant activity of olive oil seems to play the key role. In addition, some compounds from olive oil, particularly polyphenols, show potent effects on cell signalling via modulation of enzymes activity and protein expression, therefore their use became interesting not only in prevention, but also in the treatment of certain diseases.

Key words: olive oil, polyphenols, antioxidants, healthy diet

OLIVO – FONTE DI SOSTANZE FARMACODINAMICAMENTE ATTIVE

SINTESI

L'olivo ed i suoi prodotti vengono riconosciuti come un'importante componente di una dieta salutare. L'aumento del consumo dell'olio d'oliva è implicato nella riduzione delle malattie cardiovascolari, dell'artrite reumatica e di varie forme di cancro. Nella prevenzione di tali malattie, l'attività antiossidante dell'olio d'oliva ha una funzione chiave. Alcuni composti dell'olio d'oliva, in particolare i polifenoli, hanno un potente effetto sulla segnalazione cellulare tramite modulazione dell'attività enzimatica e dell'espressione proteica, pertanto il loro utilizzo è diventato interessante non solo nella prevenzione ma anche nella cura di alcune malattie.

Parole chiave: olio d'oliva, polifenoli, antiossidanti, dieta salutare

INTRODUCTION

Olive and its products have been recognized as an important component of a healthy diet. The number of reports, describing the beneficial properties of olive oil, has dramatically increased in the last couple of years, and recent data have suggested that olive oil has more health benefits that previously thought. The growing popularity of the Mediterranean diet is due to several epidemiological studies that show the lowest incidence of coronary heart disease and certain cancers, *e.g.*, breast and colon cancers in the Mediterranean basin. It has been suggested that this is largely due to the relatively safe and even protective dietary habits of this area, where olive oil represents the principal source of fat in a diet (Hertog *et al.*, 1995; Keys, 1995; Visioli *et al.*, 2005).

Olive oil's vital components, monounsaturated fatty acids and antioxidant and anti-thrombotic substances are attributed with its protective effects against, among others, atherosclerosis and other cardiovascular diseases (Manna *et al.*, 2004; Dell'Agli *et al.*, 2006; Perona *et al.*, 2006), diabetes (Al-Azzawie *et al.*, 2006), certain cancers (Owen *et al.*, 2004; Visioli *et al.*, 2004; Hashim *et al.*, 2005), inflammation diseases (Pattison *et al.*, 2004; Puel *et al.*, 2004; Beauchamp *et al.*, 2005) and age related cognitive decline (Wahle *et al.*, 2004).

This review is focuses on biologically active constituents of olive and particular olive oil and their importance for human health. The molecular mechanisms involved in pharmacologically effects of certain compounds are highlighted.

THE COMPOSITION OF OLIVE OIL

Olive oil is obtained from the drupes of olive tree that is best grown between the 30^0 and the 45^0 parallel. Accordingly, the Mediterranean countries supply more than 95% of the world olive oil production that is around 2.000.000 tons/year (Visioli *et al.*, 2002). Depending on its chemical properties, organoleptic characteristics and its degree of acidity, olive oil is classified into different grades (EEC Council regulations, 1991). From this classification, the most valuable is the extra-virgin oil, obtained from intact olives that are quickly processed and cold-pressed. In this way, activation of cellular lipases and degradation of the triglycerides is minimized (Visioli *et al.*, 2002).

The composition of olive oil is primarily saponifiable glyceridic compounds as triglycerides (Montedoro, 1972), where the oleic acid, a monounsaturated acid (18:1n-9) represents 56 to 84% of total fatty acid, while linoleic acid (18:2n-6) is present in 3 to 21% (Boskou, 2000; Butinar *et al.*, 2004).

The biological effects of monounsaturated fatty acids in olive oil on circulating lipids and lipoproteins in human body are somewhat controversial (Visioli *at al.*, 2002): while the major effects of high monounsaturated fatty acids intakes on serum cholesterol are generally attributed to the associated replacement of saturated fatty acids (Hegsted *et al.*, 1993; Gardner *et al.*, 1995), some studies attributed a direct, although modest cholesterol-lowering effect to monounsaturated fatty acids alone, when they equicalorically replace carbohydrates. Furthermore, monounsaturated fatty acids increase the levels of the protective high-density lipoprotein (HDL) more than polyunsaturated when these two classes of fatty acids replace carbohydrates in the diet (Mensink *et al.*, 1992).

Unsaponifiable compounds represent 0.5 to 1.0% of constituents of minor fraction in olive oil. Among minor constituents of virgin olive oil, there are vitamins such as α- and γ-tocopherols (around 200 ppm) and β-carotene, phytosterols, pigments, terpenoids, flavonoids such as luteolin and quercetin, squalene, and more than 30 different phenolic compounds (Montedoro, 1972; Butinar *et al.*, 1999; Boskou, 2000), some of them with potent antioxidant activity, which is important in the prevention of cardiovascular and cancer diseases and inflammation (Boskou, 1996) (Tab. 1).

The amount of phenolic compounds in olive oil depends on several factors, including cultivar, degree of maturation, possible infestation by the olive fly *Dacus olea*, and climate (Boskou, 2000; Butinar *et al.*, 2000a), and usually decreases with over-maturation of olives (Visioli *et al.*, 2002).

The three phenolic compounds in highest concentration in olive oil are the glycoside oleuropein, hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and tyrosol. These three compounds are related structurally. Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol possesses an extra hydroxy group in the *meta* position (Tuck *et al.*, 2002). Oleuropein is an ester composed of hydroxytyrosol and elenolic acid. Oleuropein is the major phenolic compound in olive drupes, whereas hydroxytyrosol is the major phenolic component in olive oil (Amiot *et al.*, 1996). As the olive drupe matures, the concentration of oleuropein decreases, while hydroxytyrosol, a hydrolysis product of oleuropein, increases (Cimato *et al.*, 1990; Ryan *et al.*, 1999).

Tab. 1: The chemical composition of olive oil.
Tab. 1: Kemična sestava oljčnega olja.

Olive oil	Subfraction	Component
Major fraction (98–99%, saponifiable)	triglycerides	oleic acid, linoleic acid
Minor fraction (1–2%, unsaponifiable)	hydrocarbons	squalene, β-carotene, polycyclic aromatic hydrocarbon
	sterols	β-sitosterol, campesterol, Δ7-stigmasterol, brassicasterol
	terpenic dialcohols	erythroidol, uvaol
	tocopherols	α-tocopherol, β-tocopherol, γ-tocopherol, Δ-tocopherol
	phenolic compounds	tyrosol, hydroxytyrosol, caffeic acid, oleuropein
	others	flavour components

PHARMACODYNAMICALLY EFFECTS OF OLIVE OIL CONSTITUENTS

Recent studies showed that certain olive oil constituents exert strong pharmaco-dynamic effects in human body. They have potent modulatory effect on cell signalling and became interesting not only in prevention, but also in the treatment of certain diseases. So far, several biologic activities of olive oil compounds have been demonstrated, like: scavering of superoxide and other reactive oxygen substances (ROS) (Le Toutour *et al.*, 1992; Aeschbach *et al.*, 1994; Manna *et al.*, 1997) inhibition of low-density lipoprotein (LDL) oxidation (Scaccini *et al.*, 1992; Visioli *et al.*, 1994), inhibition of apoprotein derivatization (Visioli *et al.*, 1995), reduced thromboxan B_2 and leukotriene B_4 production by activated leukocytes, inhibition of platelet aggregation and thromboxane generation (Petroni *et al.*, 1995), inhibition of peroxynitrite-induced DNA damage and inhibition of peroxynitrite-induced tyrosine nitration (Deiana *et al.*, 1999), inhibition of bacterial growth and activity, and decreased isoprostane excretion in humans and in sidestream smoke-exposed rats (Tuck *et al.*, 2002), and others, like scavering of hypochlorus acid, increased nitric oxide production by mouse macrophages, cytostasis, hypotensive action, and increased plasma antioxidant capacity (for review see Visioli *et al.*, 2002).

Antioxidant activity of olive oil constituents

Antioxidant activity of olive oil constituents, particularly polyphenols, seems to play a key role in the beneficial effect in several diseases, like cardiovascular diseases, cancer and inflammation. The main mechanism by which the components of olive oil express their antioxidant activity is inhibition and/or scavenging of ROS, which can activate different signalling pathways that lead to progression of disease. In the excess of ROS, they can also react with different cellular constituents and cause cell damage. ROS are produced during normal metabolism or after oxidative processes and include superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Voetsch *et al.*, 2004). In the increased production of O_2^- and H_2O_2, they react rapidly with nitric oxyde (NO) to form peroxynitrite ($OONO^-$), thus inactivating NO and leading to different physiological dysfunctions (Perona *et al.*, 2006) (Fig. 1).

The formation of ROS is balanced by a range of antioxidant defences, but the excess can overwhelm these systems and leads to oxidative stress, which importantly contributes to the development of certain diseases. Several constituents in olive oil potently modulate the ROS production. Oleic acid and β-sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. β-Sitosterol may also enhance superoxyde dysmutase activity, hence decreasing O_2^- levels. This reduction has also been observed for the terpenoid oleanolic acid, although the mechanism is not presently known. Tocopherols and phenolic compounds are potent antioxidants that may help reduce lipid peroxidation and scavenge intracellular ROS and free NO, reducing the formation of $OONO^-$. ROS can activate the nuclear factor κB (NFκB), which is then translocated into the nucleus, where it binds to recognition sequences in DNA to induce gene expression. This mobilization of NFκB is blocked by α-tocopheryl succinate but not by α-tocopherol. In contrast, phenolic compounds have been proposed to act blocking the formation of NFκB/DNA binding complexes. NFκB modulates the expression of cytokines, enzymes lipoxygenase (LOX) and cycloxygenase (COX), thereby affecting the levels of adhesion molecules and eicosanoids. However, some of the minor compounds of olive oil may act directly on these enzymes and cytokines. LOX and COX activities are inhibited at different points by phenolics and triterpenoids, whereas interleukin-1β (IL-1β) expression is inhibited by phenolics and tocopherols, contributing to protect the endothelium against vasoconstriction, platelet aggregation and monocyte adhesion. Vasodilatation is also suggested to be enhanced by oleuropein and oleanolic acid through an increase in the production of NO (Tab. 2) (for review see Perona *et al.*, 2006).

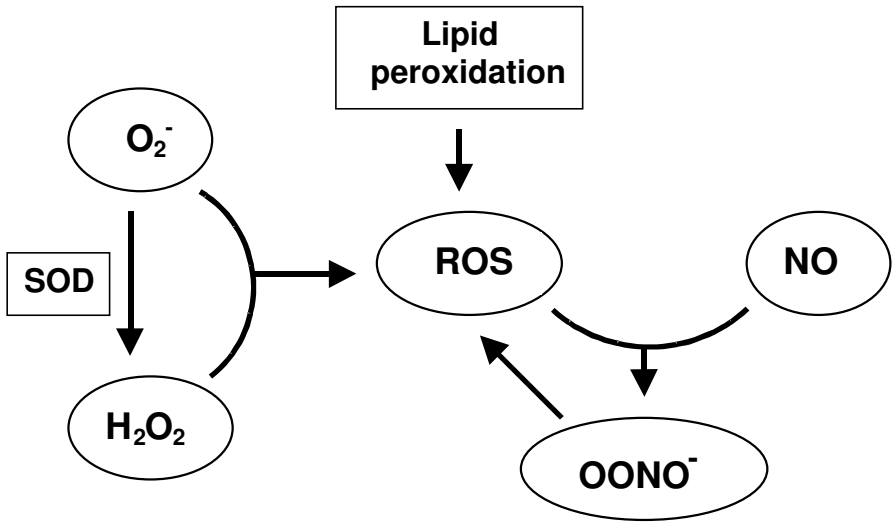

Fig. 1: Different biochemical pathways of reactive oxygen substances (ROS) production in the cell (SOD-superoxyde dysmutase).
Sl. 1: Različne biokemične poti nastanka reaktivnih kisikovih spojin (ROS) v celici (SOD-superoksid-dismutaza).

Tab. 2: Influence of olive oil constituents on biochemical processes in the cell.
Tab. 2: Vpliv sestavin oljčnega olja na biokemične procese v celici.

Olive oil constituent	Mechanism of action
oleic acid	Inhibition of ROS production
polyphenols	Reduction of lipid peroxidation Scavenging intracellular ROS Reduction of OONO- formation Reduction of NFκB/DNA binding complexes formation Inhibition of LOX and COX Inhibition of IL-β expression Increasing NO production
tocopherols	Scavenging intracellular ROS Reduction of OONO- formation Block of NFκB mobilisation to the nucleus Inhibition of IL-β expression
β-sitosterol	Inhibition of ROS production Activation of superoxyde dismutase
terpenoids	Inhibition of LOX and COX

The effects of olive oil constituents in cardiovascular diseases

Olive oil constituents show complex benefit effects in prevention of cardiovascular diseases. Cardiovascular diseases risk factors, like hypercholesterolemia (Ohara *et al.*, 1993; Stokes *et al.*, 2002), diabetes mellitus (Guzik *et al.*, 2002) and hypertension (Just, 1997; Kerr *et al.*, 1999), are all strongly related to increased production of ROS, inactivation of NO and endothelial dysfunction. ROS can also react with polyunsaturated fatty acids contained in lipoproteins in the vessel wall, initiating lipid peroxidation. The hydroperoxides formed in this process can in turn react with NO to form $OONO^-$, inactivating NO, and directly decrease the endothelial synthesis of NO (Chin *et al.*, 1992).
Monounsaturated fatty acids in olive oil increase the levels of the protective HDL and improve therefore lipoprotein profile in the body. Beside this, they reduce the thrombogenic-atherogenic process by various actions on arterial thrombus formation, such as decreased monocyte adhesion, increased fibrinolysis and decreased arte-

rial pressure. Additionally, the antioxidant substances found in olive oil (*i.e.* tocopherols, polyphenols) could influence atherogenesis by inhibition of LDL-cholesterol oxidation, protection against free radicals and their toxic effects, inhibition of platelet aggregation and thromboxane generation, stimulation of anti-inflammatory agents and increased nitric oxide production (Perona *et al.*, 2006).

The effects of olive oil constituents in inflammation

Certain compounds from olive oil have been shown to modulate immune function that might be interesting in the treatment of inflammatory processes, associated with the immune system like rheumatoid arthritis (Pattison *et al.*, 2004; Puel *et al.*, 2004; Beauchamp *et al.*, 2005).

In the inhibition of inflammatory processes by olive oil constituents, the reduction of intracellular ROS production is important, since ROS enhances transcriptional activity via activation of NFκB that modulates the expression of certain pro-inflammatory cytokines like IL-1β, IL-6 and tumour necrosis factor α. Beside this, reduction of ROS production inhibits LOX and COX transcription and activity. Consequently, the cyclooxygenase and lipoxigenase pathway of arachidonic acid metabolism, and the production of prostaglandins and other inflammatory mediators are diminished. Some of the minor compounds of olive oil may act directly on selected enzymes and cytokines, involved in inflammation; LOX and COX activities are inhibited at different points by phenolics and triterpenoids, whereas IL-1β expression is inhibited by phenolics and tocopherols (Beauchamp *et al.*, 2005; Perona *et al.*, 2006).

The effects of olive oil constituents in cancer

In the ethiopathogenesis of cancer disease, there are several factors contributing to the development and progress of the disease. Among them, oncogenic substances, oxidative stress and angiogenesis play an important role.

Several epidemiological studies show beneficial effects of olive oil constituents in prevention of cancer diseases (Owen *et al.*, 2004; Visioli *et al.*, 2004; Hashim *et al.*, 2005). Olive oil constituents inhibit production of ROS and have protective role in conditions, where the excess production of ROS leads to oxidative stress and causes oxidative cell damage. Certain compounds from olive oil also inhibit the expression of pro-oncogenic substances (Nelson, 2005). Recent studies showed inhibitory effect of olive oil polyphenols on proliferation of human promyelocytic leukemia cell by inducing apoptosis and differentiation (Fabiani *et al.*, 2006). Beside this, it was found strong anti-angiogenic effect of oleuropein, which causes irreversible changes in cancer cells, preventing their replication, motility and invasiveness (Hamdi *et al.*, 2005).

These effects may explain the cancer-protective effects of the olive-rich Mediterranean diet and may also have important therapeutic implications in the treatment of cancer disease.

CONCLUSION

Epidemiological studies show that populations consuming a predominantly plant-based Mediterranean-style diet exhibit lower incidences of chronic diseases than those consuming a northern European or North American diet. Although total fat intake in Mediterranean populations can be higher than in other regions, the greater proportion is derived from olive oil and not animals. Increased olive oil consumption is implicated in a reduction in cardiovascular diseases, rheumatoid arthritis and, to a lesser extent, a variety of cancers. Olive oil intake also has been shown to modulate immune function, particularly the inflammatory processes associated with the immune system (Wahle *et al.*, 2004).

Typical of olive oil is combination of high oleic acid content and content of a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, and tyrosol. In the prevention of diseases, strong antioxidant activity of olive oil seems to play the key role. In addition, some compounds from olive oil, particularly polyphenols, showed potent effects on cell signalling via modulation of enzymes activity and protein expression, therefore their use became interesting not only in prevention, but also in the treatment of certain diseases.

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POVZETEK

Oljka in izdelki iz oljke so že dolgo znani kot pomembna sestavina zdrave prehrane. Dokazani so bili številni koristni učinki sestavin oljčnega olja, plodov in listov oljke. S količinskega vidika so glavna komponenta oljčnega olja umiljivi triacilgliceroli, medtem ko manjši del sestavljajo neumiljive snovi, kot so steroli, terpenski alkoholi, tokoferoli, polifenoli in druge spojine. Večina sestavin (oleinska kislina, polifenoli, tokoferoli, β-sitosterol, terpenoidi) ima močan modulatoren učinek na celično signaliziranje, zato so postale zanimive za preprečevanje in zdravljenje nekaterih bolezni.

Najbolj preučevan učinek je zaščitna vloga oljčnega olja pri boleznih srca in ožilja, kjer imata ključno vlogo moteno delovanje žilnega endotelija in razvoj ateromatoznih plakov. Glavni mehanizem delovanja, po katerem sestavine oljčnega olja vplivajo na delovanje žilnega endotelija, je zaviranje delovanja reaktivnih kisikovih spojin in lovljenje prostih radikalov. To je posledica zaviranja nastanka reaktivnih kisikovih spojin pa tudi aktivacije encima superoksid-dismutaze. Poleg tega oleuropein in oleanolna kislina s povečanjem nastanka NO povzročata tudi razširitev žil.

Polifenoli so učinkoviti tudi pri zaviranju aktivnosti nekaterih pro-vnetnih citokinov, kot je npr. IL-1β, ter encimov ciklo-oksigenaze in lipo-oksigenaze. Ti procesi so posredovani najmanj po dveh poteh. Prvo pot predstavlja zaviranje mobilizacije NFκB, ki je pomembna za izražanje genov številnih pro-vnetnih citokinov in encimov. Poleg tega delujejo polifenoli in tokoferoli tudi kot lovilci prostih radikalov in s tem zavirajo nastanek vnetnih mediatorjev iz arahidonske kisline. Ker polifenoli tako neposredno vplivajo na nastanek vnetnega in/ali imunskega odgovora, so postali zanimivi za zdravljenje bolezni, kot je npr. revmatoidni artritis, ter degenerativnih boleznih živčnega sistema, kakršna je npr. Alzheimerjeva bolezen. Nekatere sestavine oljke, zlasti tokoferoli, skvaleni in polifenoli, pa so zaradi antioksidativnega in protitumorskega učinka zanimive tudi za preprečevanje nastanka raka.

Ključne besede: oljčno olje, polifenoli, antioksidanti, zdrava prehrana

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PRISPEVEK K PREGLEDU TAKSONOV IZ OBLIKOVNEGA KROGA ŠIROKOLISTNE MOČVIRNICE (*EPIPACTIS HELLEBORINE* S.L.)

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IZVLEČEK

Prispevek predstavlja pregled alogamnih in avtogamnih taksonov iz oblikovnega kroga širokolistne močvirnice – Epipactis helleborine*. Opisana sta dva nova taksona za floro Slovenije:* Epipactis helleborine *subsp.* orbicularis *in* Epipactis leptochila *subsp.* neglecta*. Hkrati sta predstavljeni tudi dve nedokončno opredeljeni močvirnici, prva iz območja Lendave, druga je provizorično kategorizirana kot* Epipactis helleborine *subsp.* minor*. Izdelan je ključ za določanje močvirnic iz oblikovnega kroga širokolistne močvirnice z območja Slovenije.*

Ključne besede: *Epipactis,* kukavičevke, pregled taksonov, ključ za določevanje, Slovenija

CONTRIBUTO ALLA REVISIONE DEI TAXA DEL GRUPPO DI ELLEBORINE COMUNE (*EPIPACTIS HELLEBORINE* S.L.)

SINTESI

Il contributo offre una revisione dei taxa autogami e allogami del gruppo di Elleborine comune - Epipactis helleborine*. Vengono descritti due nuovi taxa per la flora della Slovenia:* Epipactis helleborine *subsp.* orbicularis *ed* Epipactis leptochila *subsp.* neglecta*. Vengono inoltre presentate due nuove elleborine non ancora definite, la prima proveniente dalla regione di Lendava, la seconda categorizzata provvisoriamente come* Epipactis helleborine *subsp.* minor*. È stata elaborata una chiave di determinazione del gruppo delle elleborine del territorio sloveno.*

Parole chiave: *Epipactis,* orchidee spontanee, revisione dei taxa, chiave di determinazione, Slovenia

UVOD

Predstavitev širokolistne močvirnice

Ta najbolj razširjena vrsta močvirnic se pojavlja v zmernem klimatskem pasu Evrope in v Skandinaviji, najdemo pa jo še v centralni Sibiriji, od Kavkaza do Pakistana in Turkestana. Nedavno so jo zabeležili v Kanadi in Ameriki, vendar prevladuje mnenje, da je bila tja prinesena (Reinhard *et al.*, 1991). Raste v različnih gozdovih z bogato hranljivo podlago do višine 1600 m. V Franciji so jo našli še na višini 2300 m, najviše rastočo so odkrili v Sikkimu na višini 4000 m. Širokolistna močvirnica nima posebnih zahtev, uspeva v gozdovih, na gozdnih jasah, ob poteh, na gozdnem obrobju in med grmovjem, vendar raje izbira senčno lego. Do 80 in 90 cm visoka kukavičevka ima široko ovalne stebelne liste, ki se više ob steblu nekoliko zmanjšajo, postanejo bolj zašiljeni in so na zgornji strani temnozeleno bleščeči. Barva cvetov ni najbolj zanesljivo merilo, zato pa je v cvetni zgradbi dobro viden razvit kljun (rostellum), ki ostane dolgo funkcionalen. Širokolistna močvirnica sodi v krog močvirnic, ki se razmnožujejo (obligatno) alogamno (Sl. 12, 13).

Opis problematike

Določanje širokolistne močvirnice postane zapleteno, kadar je njen habitus spremenjen in kadar je ne najdemo v senčnem gozdu. V ključu "Naše orhideje" Jogan (2000) ugotavlja, da težavnost določanja močno narašča v drugem delu ključa, zlasti zaradi problematične skupine okoli širokolistne močvirnice. Gre za močvirnice, ki imajo drugačen habitus in ki cvetijo na svetlem, polsuhem zemljišču, na sončnem obrobju gozda in se razlikujejo od tipične širokolistne močvirnice. Te močvirnice cvetijo približno 10 do 14 dni prej. Talna podlaga je skromna, plast zemlje, na kateri rastejo, je tanjša. Stebelni listi so svetlo zeleni, manj ovalni, medtem ko se cvetovi očitno ne razlikujejo od nominalne vrste. Izredna variabilnost povzroča še danes razhajanja v mnenjih, kam sodi posamezna močvirnica, ki je v mnogočem podobna širokolistni močvirnici. Na podlagi opazovanj in primerjanj so v novejšem obdobju opisali nove vrste ali podvrste, ki sodijo v krog širokolistne močvirnice (Presser, 2002; Redl, 2003; Perko, 2004). Pri nas sta nekatere objavila Jogan (2000) in Ravnik (2002). Še obsežneje o njih poročajo drugi avtorji, ki sodijo predvsem v nemško govoreči prostor (Delforge, 1995; Presser, 2002; Redl, 2003; Perko, 2004). Na drugi strani pa nekateri botaniki sodijo, da je razlika v habitusu odsev prilagajanja širokolistne močvirnice drugačnim razmeram v okolju ter da ne gre za posebne taksone. Problem je v tem, da je določanje močvirnic, ki se razmnožujejo alogamno, enostavnejše kakor določanje avtogamnih močvirnicah, kjer morfološke kriterije težko definiramo.

Namen prispevka je podati pregled na območju Slovenije najdenih taksonov, ki zaradi svoje podobe sodijo v krog širokolistne močvirnice. Hkrati posredujemo nahajališča, ki smo jih obiskovali v zadnjih letih. Dodajamo na novo oblikovani dihotomni ključ za določanje desetih močvirnic, ki jih omenjamo v prispevku.

METODE

Na osnovi rednih vsakoletnih opazovanj močvirnic na terenu smo razmeroma dobro spoznali pojavljanje močvirnic ter njihove morfološko ustaljene znake. Če so pri tem nastale različice, smo te primerjali z ugotovitvami iz prejšnjih sezon. Vsem je skupna zelena podoba celotne zgradbe močvirnice, barva cvetov od zeleno-belih prek rožnato-rdečkastih do rumenkastih odtenkov in velikost cele rastline. Časovno se obdobje rasti in cvetenja močvirnic, o katerih razpravljamo, prične konec junija in traja še meseca avgusta. Posamezne vrste smo fotografirali na terenu, vzorce pa smo fotografirali kasneje doma zaradi podrobnosti, ki jih ni bilo mogoče napraviti na terenu. Hkrati smo z risbo skušali približati morfološke posebnosti, ki jih fotografsko ni možno (zanesljivo) prikazati. Risbe so v nadaljnjem prikazane za taksone, kjer je to potrebno zaradi razumevanja problematike. Preglednejšo razvrstitev močvirnic smo dosegli z razdelitvijo v alogamne in avtogamne taksone. Nomenklaturo smo uporabili iz Male flore Slovenije (MFS). Za vrste ali podvrste močvirnic, ki jih MFS ne obravnava, povzemamo nomenklaturo po Perku (2004) in Redlu (2003).

REZULTATI IN DISKUSIJA

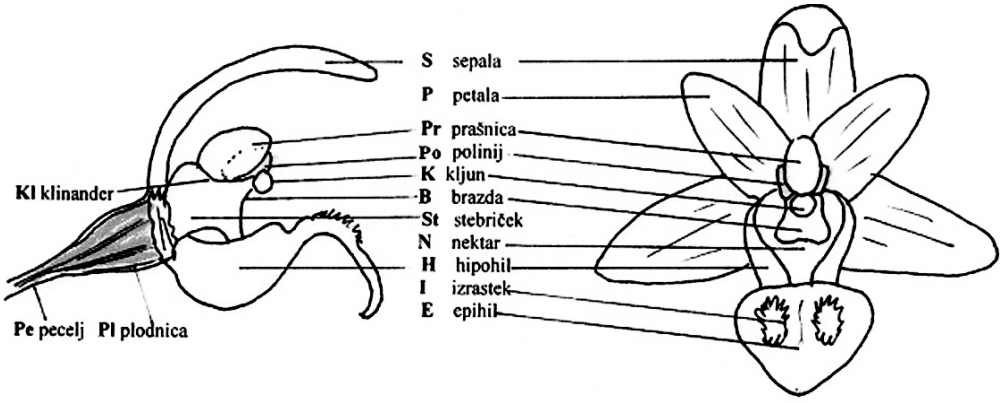
Razdelitev

V ožji krog širokolistne močvirnice sodijo sledeči največkrat (fakultativno) **alogamni** taksoni:

- *Epipactis helleborine* subsp. *orbicularis* (Richter) Klein, kratkolistna močvirnica
- *Epipactis latina* (Rossi & E. Klein) B. & H. Baumann, italijanska močvirnica
- *Epipactis leutei* Perko & Robatsch, leutejeva močvirnica

In še šest **avtogamnih** taksonov, ki sodijo v širši krog širokolistne močvirnice. Med njimi so posamezni taksoni, ki se v določenih razmerah razmnožujejo tudi alogamno.

- *Epipactis muelleri* Godfery, müllerjeva močvirnica
- *Epipactis leptochila* (Godfery) Godfery, ozkoustna močvirnica
- *Epipactis leptochila* subsp. *neglecta* H. Kümpel, prezrta močvirnica



Sl. 1: Morfološka zgradba cveta močvirnic: stranski pogled (levo) in čelni pogled (desno).
Fig. 1: Morphological structure of the Helleborines' flower: lateral view (left) and frontal view (right).

– *Epipactis greuteri* H. Baumann & Künkele, greuterjeva močvirnica
– *Epipactis pontica* Taubenh. [*E. helleborine* (L.) Crantz subsp. *pontica*
– (Taubenheim) Sundermann], pontska močvirnica
– *Epipactis nordeniorum* K. Robatsch, nordenova močvirnica

Ključ za določanje močvirnic iz oblikovnega kroga širokolistne močvirnice

Morfološka zgradba cveta močvirnic in posebej še videz stebrička nam lahko pomagata določiti vrsto močvirnice (Sl. 1). Na stranski in čelni sliki so prikazani vsi elementi, ki jih vidimo pri alogamnih močvirnicah. Pri avtogamnih močvirnicah lahko kljun manjka ali pa je slabo razvit. Pri teh močvirnicah prašnica in polinij pogosto segata čez rob stebrička nad brazdo (Sl. 2). Oceniti moramo, ali se vidi klinander (clinadrium). Klinander je del stebriča, ki se nadaljuje v polprašnico. V njej je pelod v obliki polinija. Klinander je skledast zaključek na vrhu stebriča in ga pri večini močvirnic ne vidimo, ker ga zasedata grudici peloda. Če pa sta grudici peloda potisnjeni naprej kot pri ozkoustni močvirnici, lahko vidimo majhen prostor, ki daje videz prehoda, če stebriček gledamo z lupo od strani.

Izhajamo iz podrobnega opisa **širokolistne močvirnice [*Epipactis helleborine* (L.) Crantz subsp. *helleborine*]**. Temu nato sledi določevalni ključ za taksone iz oblikovnega kroga širokolistne močvirnice:

Najnižji stebelni list je pri širokolistni močvirnici razmeroma majhen, tako širok kot ovalen. Naslednji je največji. Višje spiralno nameščeni listi so bolj podolgovati. Listi so temnozeleni, na zgornji strani bleščeči. Socvetje bogato. Cvetovi odprti, gledajo naravnost naprej ali so rahlo viseči. Barve so lahko zelo spremenljive, navadno zelenorožnata. Sepalni listi so zelenorožnati, krilati z žilami, podobno kot krajša petalna lista, ta sta belorožnata. Medena ustna je brez ostroge, hipohil ima zunanjo stran svetlozelenkasto, nektar je rjav ali temnordeče rjav. Prehod v epihil je srednje širok, žlebast. Epihil (lupa!) je prej širši kot daljši, srčast z dvema izrastlinama (Calli). Konica epihila je zavihana navzdol. Stebriček (lupa!): prašnica sedeča, svetlorumena. Dve čvrsti grudici peloda, brazda pravokotna, postavljena nekoliko poševno in naprej. Kljun bel, dobro razvit, funkcionalen, ima lepljivo ploščico. Cvetovi alogamni. Plodnica približno 10 mm, nekoliko viseča.

Sl. 2. Zgradba stebričkov pri posameznih močvirnicah. Vrste si sledijo od leve proti desni: *E. helleborine*, *E. leptochila*, *E. mülleri*, *E. greuteri*. ***Legenda: P - prašnica, K - kljun, B - brazda, Kl - klinander, Po - polinij, S – stebrič.***
Fig. 2. The structure of gynostegium of individual *Epipactis*. ***From left to right:*** *E. helleborine*, *E. leptochila*, *E. mülleri*, *E. greuteri*. ***Legend: P – anther, K – rostellum, B – stigma, Kl – clinadrium, Po - pollinia, S – Ggnostegium.***

Ključ za vrste močvirnic iz oblikovnega kroga širokolistne močvirnice

1 Cvetovi štrleči do viseči, zvonasti, priprti, zaprti, hipohil tvori skledasto obliko, nektar v hipohilu temnejši od drugih barv cveta. Stebelni listi okrogli, ovalni do suličasti, sedeči, listne žile vzporedne. Plodnica pecljata. Epihil z nagubanima izrastkoma. Epihil srčast. Prehod iz hipohila v epihil je žlebast. Steblo je zeleno, spodaj ima včasih rahlo rdeče vijolični odtenek ..... ***Epipactis helleborine* subsp. *helleborine***
1* Stebelni listi enako dolgi ali daljši, kot so internodiji. Cvetovi belo-zelenkasti, zelenkasti ali od rdeče-rjavkastih do vijoličnih odtenkov. Plodnica gladka, morda komaj dlakavo poraščena 2

2 Cvetovi **alogamni**. Pri svežih cvetovih je kljun na zgornjem robu stebriča kot kroglasta tvorba. Je dobro viden. Grudice peloda kompaktne, se ne drobijo 3
2* Cvetovi **avtogamni**. Kljuna ni ali ni funkcionalen. Grudice peloda se drobijo, padajo na brazdo .. 5, 6, 7, 8, 9, 10

3 Steblo zeleno, kvečjemu na dnu nekoliko vijolično obarvano. Listi zeleni brez vijoličnih odtenkov. Cvetovi brez izrazitih vijoličnih in rdeče-rjavkastih delov, petalni listi so kvečjemu nežno rožasto obarvani. Listi kratki, široko-ovalni, okroglasti, ob steblu izrazito obrnjeni navzgor, kot škrniclji. Epihil belkast, največkrat nežno rožnat, po dolžini epihila v sredini z rjavkasto-vijolično črto ..... ***Epipactis helleborine* subsp. *orbicularis***
3* Cvetovi največkrat z vijolično in rdečerjavimi deli. Listi jajčasti-suličasti, stojijo horizontalno od stebla ali nekoliko poševno navzgor 4

4 Širina epihila je malo manjša od njegove dolžine. Prehod iz epihila v hipohil je ozek. Steblo je v spodnji polovici močno rdeče-vijolično obarvano. Steblo tu in tam malo upognjeno ..... ***Epipactis leutei***
4* Stebelni listi obrnjeni navzgor**,** robovi valoviti, petalna lista intenzivno rdečkasta, hipohil belo-škrlaten, epihil srčast, kljun funkcionalen ..... ***Epipactis latina***

5 Širok prehod iz hipohila v epihil, epihil je širši kot daljši, bolj ali manj zavihan navzdol. Stebelni listi togi, na robovih valoviti, prej suličasti, srpasti, na koncu zavihani navzdol. Prašnica potisnjena močno naprej, pelodne grudice se drobijo nad navpično položeno brazdo ..... ***Epipactis muelleri***

6 Epihil je razločno daljši od njegove širine, zašiljen, ni zavihan. Prehod iz hipohila v epihil v obliki črke V. Klinander izražen (lupa!). Listi jajčasto-suličasti, rahlo povešeni ..... ***Epipactis leptochila* subsp. *leptochila***

7 Zašiljeni epihil je zavihan nazaj, rob epihila rahlo valovit ..... ***Epipactis leptochila* subsp. *neglecta***

8 Stebelni listi na videz uveli, temno-zelenkasti, na robovih valoviti, cvetni pecelj precej podolgovat, cvetovi viseči, zvonasti, epihil nekoliko daljši od njegove širine – bel, svetlo zelen ali roza nadahnjen, kljun pri svežih cvetovih ni funkcionalen ..... ***Epipactis greuteri***

9 Razmeroma majhni, zeleno rumenkasti cvetovi. Cvetni listi so zvonasti, priprti. Nektar v hipohilu zelen, kasneje temnorjav, prehod v epihil skledast. Izrastlini na belem epihilu zelenkasti, kasneje rahlo rožnate barve, epihil rombast, na vrhu zaokrožen, kljun ni funkcionalen ..... ***Epipactis pontica***

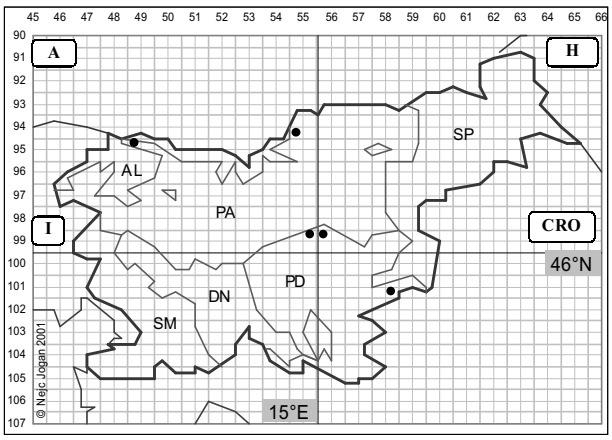
10 Majhna močvirnica, veliki jajčasto okrogli stebelni listi, cvetovi odprti, po nekaj dneh se zaprejo, zunanji cvetni listi zunaj zelenkasti, znotraj rumeno rožnato nadahnjeni. Notranji cvetni listi močneje rožnato obarvani. Nektar v hipohilu rdečkasto-rjav, prehod v epihil zelo ozek. Epihil srčast, rožnato rdečkasti izrastlini se na sredini združujeta. Brazda kvadratasta, kljun ni funkcionalen. Plodnica velika in okrogla ..... ***Epipactis nordeniorum***

Kratek pregled posameznih taksonov

Alogamni taksoni

Epipactis helleborine subsp. *orbicularis* (Richter) Klein, kratkolistna močvirnica

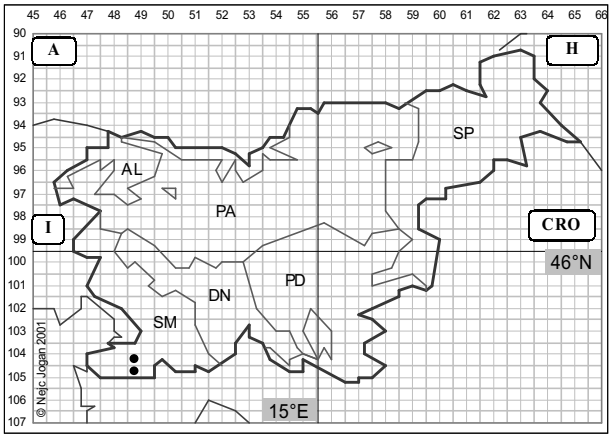
Podobnost s širokolistno močvirnico se konča pri stebelnih listih, ki so skoraj enakomerno veliki, žličasti, lahko zmerno suličasti in ob steblu obrnjeni navzgor. Nastavek lista objema steblo in ima na spodnjem delu bel rob. So lahko dvovrstni. V višino meri do 60 cm, morda tudi več. Socvetje je bogato, cvetovi so lepo odprti in izredno podobni širokolistni močvirnici. Medena ustna kaže ozek prehod iz hipohila v epihil, na njem je po sredini večkrat vidna vijolična črta med levo in desno stranjo. Kratkolistna močvirnica cveti 10 dni do dva tedna pred širokolistno močvirnico. Našli smo jo ob poti pod borovimi drevesi nad Mežico proti Jankovcu v višini 900 mnv (Lipovšek), na Gorjancih (Kosec), v dolini Sopote in na pobočju pod Podkumom (Klenovšek), na Srednjem Vrhu nad Martuljkom (Lipovšek) (Sl. 3). Verjetno je bolj pogosta, toda ker je zelo podobna širokolistni močvirnici, lahko ostane neprepoznavna (Sl. 14, 15).



***Sl. 3: Fitogeografska razširjenost vrste** E. helleborine **subsp.** orbicularis.*
***Fig. 3: Phytogeographic distribution of the species** E. helleborine **subsp.** orbicularis.*

Epipactis latina (Rossi & E. Klein) B. & H. Baumann, italijanska močvirnica

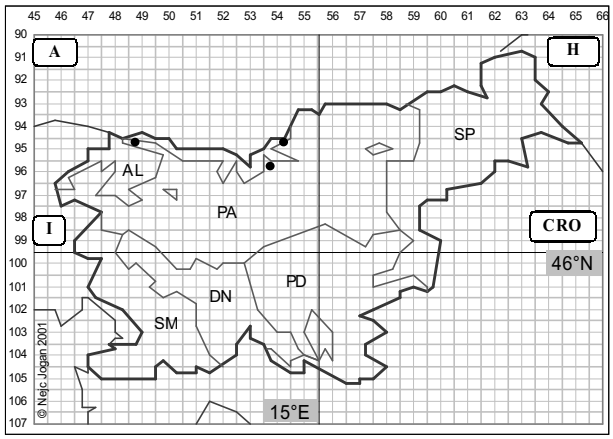
Je na pogled visoka, do 80 cm visoka močvirnica z rdečerožnatimi perigonovimi listi. Njena podobnost s širokolistno močvirnico je tako izrazita, da jo nekateri avtorji sploh ne omenjajo kot posebno vrsto (Presser, 2002). Cveti zgodaj, v drugi polovici junija. Pri nas raste pod Kraškim robom na več mestih blizu Podpeči in Zazida ter ob cesti Gračišče-Brezovica (A. Reichelmann, *osebno poročilo)* (Sl. 4). Ravnik (2002) jo opisuje kot samostojno vrsto (Sl. 16, 17).



***Sl. 4: Fitogeografska razširjenost vrste** E. latina.*
***Fig. 4: Phytogeographic distribution of the species** E. latina.*

Epipactis leutei Perko & Robatsch, leutejeva močvirnica

Ravnik (2002) omenja, da se od širokolistne močvirnice, ki ji je podobna, razlikuje po zgradbi listov, olistanosti in nekaterih znakih v cvetu. Razlike so v višini, leutejeva močvirnica je visoka 40 do 60 cm, in steblu, ki je od tal do spodnjih stebelnih listov rdečevijolično obarvan ter tu in tam rahlo upognjen. Stebelni listi so bolj suličasti kot ovalni razen spodnjega, ki je podoben spodnjemu pri širokolistni močvirnici, vendar je v celoti manjši. Rdečkasti perigonovi listi so vidni, kadar je močvirnica bolj na sončnem prostoru, primerki v senci pa imajo zelenkasto bele cvetove. Prehod iz hipohila v epihil je pri leutejevi razmeroma ozek, sam epihil pa je po dolžini malo daljši kot širši. Na Srednjem Vrhu nad Martuljkom cveti na višini okoli 900 mnv, našli pa smo jo tudi na Peci ob poti pred planinsko kočo na višini 1600 m (Lipovšek) in Robanovem kotu (Dolinar) (Sl. 5). Cveti v začetku avgusta (Sl. 18, 19).



***Sl. 5: Fitogeografska razširjenost vrste** E. leutei.*
***Fig. 5: Phytogeographic distribution of the species** E. leutei.*

Pri kratkolistni in leutejevi močvirnici smo opazili, da kljun ni bil pri vseh cvetovih enako dobro razvit. Pri nekaterih cvetovih je bil jasen, drugje pa je že usihal. Pri širokolistni močvirnici ta pojav ni bil nikoli tako očiten.

Avtogamni taksoni

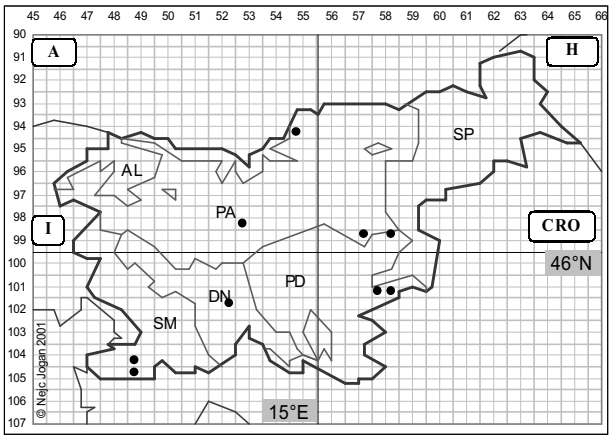
Pri naslednji skupini, ki je ločena od skupine širokolistne močvirnice zaradi avtogamnega razmnoževanja, lahko (izjemoma) pride tudi do alogamnega razmnoževanja. Meje med alogamnim in avtogamnim razmnoževanjem torej ni mogoče postavljati absolutno. Pri navedenih taksonih je avtogamija pravilo, vendar se zgodi, da pri dejavnem kljunu lahko pred njegovim razpadom pride do oprašitve z opraševalcem (npr. pri ozkoustni močvirnici). Te močvirnice povezujemo s širokolistno močvirnico iz sledečih razlogov:

– Prvi vtis "zelenkaste močvirnice" z več ali manj podobno barvo cvetov in položajem stebelnih listov (od daleč) spominja na širokolistno močvirnico. Rast-

lina v celoti ne daje enako robustnega videza (npr. pontska ali ozkoustna močvirnica).
– Opis močvirnice, ki ni tipična širokolistna močvirnica, pripelje neredko do ugotovitve, da gre pri takšnem taksonu najbolj verjetno za avtogamno razmnoževanje (npr. greuterjeva, pontska ali prezrta močvirnica) ali za (obligatno) avtogamno razmnoževanje (müllerjeva močvirnica).
– Na rastišču širokolistne močvirnice ali v neposredni bližini lahko najdemo takson(e), ki po morfologiji kaže(jo) podobnost, a se vendarle razlikuje(jo), ker: cveti(jo) pred širokolistno močvirnico, ker morda predstavlja(jo) hibrid širokolistne močvirnice, na prvi pogled kaže(jo) cvetne elemente, ki so samo podobni tistim pri širokolistni močvirnici (stebrič, kljun).

Epipactis muelleri Godfery, müllerjeva močvirnica (Sl. 20, 21)

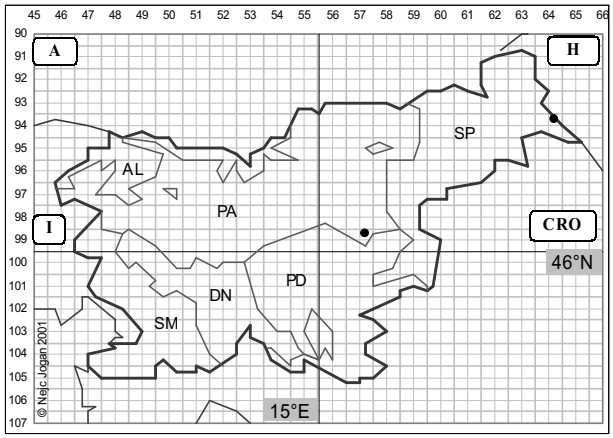
Verjetno je ta močvirnica v Sloveniji svojčas veljala za nekoliko nenavadno širokolistno močvirnico, dokler je Wraber (1979) ni prepoznal kot müllerjevo močvirnico. Glavna razlika med obema je prav gotovo oblika stebrička. Ker müllerjeva močvirnica nima kljuna, spolzi cvetni prah iz polinijev naravnost na brazdo, neredko še v času zaprtega cveta. Avtogamija je torej pri tej močvirnici pravilo. Drugi razpoznavni znak je široki prehod iz hipohila v epihil, ki ga pri drugih močvirnicah pri nas nismo opazili. Müllerjeva močvirnica cveti od sredine junija in julija od Istre do Karavank pa tudi v Kočevju, na Bloški planoti, na Lisci in Bohorju in na Štajerskem (Sl. 6). Raste posamično v manjših skupinah in jo lahko hitro spregledamo. Spodnji del medene ustne – epihil neredko kaže zašiljen konec, ki ni zavihan nazaj. Takšen videz epihila pa ima naslednja močvirnica.



***Sl. 6: Fitogeografska razširjenost vrste** E. muelleri.*
***Fig. 6: Phytogeographic distribution of the species** E. muelleri.*

Epipactis leptochila (Godfery) Godfery s.l., ozkoustna močvirnica

Za to vrsto je značilno, da je širina epihila razločno manjša od njegove dolžine. Ta meri okoli 10 mm. Majhni izrastlini na epihilu sta največkrat rožnato obarvani. Značilen je tudi prehod iz hipohila v epihil – spodnji skledasti del hipohila kaže zavihana robova, med katerima ima prehod v epihil obliko črke V. Prav posebno je zanimiva zgradba stebriča (lupa!), ki na zgornji strani kaže, gledano s strani, prehoden del pod nastavkom za prašnico; v tem primeru govorimo o izraženem klinandru. Zaradi tega je polinij 'potisnjen' naprej, kar spet omogoči drsenje cvetnega prahu na cvetno brazdo, še zlasti zato, ker kljuna ni ali pa je zakrnel. Ozkoustna močvirnica je torej avtogamna. Cveti v senčnatem bukovem gozdu na višini 780 mnv na Lisci (Sl. 7) v mesecu juliju, včasih še v avgustu (Klenovšek) (Sl. 22, 23).

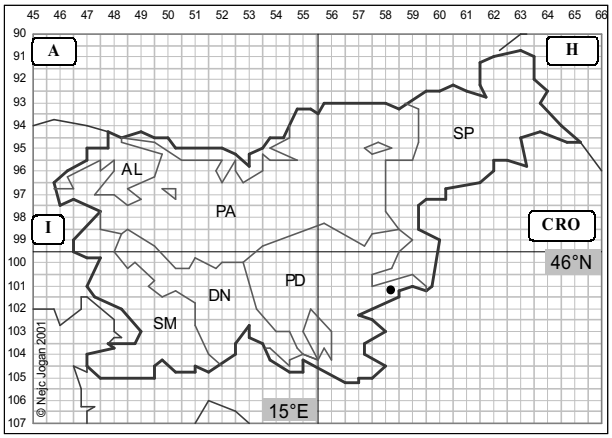


***Sl. 7: Fitogeografska razširjenost vrste** E. leptochila.*
***Fig. 7: Phytogeographic distribution of the species** E. leptochila.*

Epipactis leptochila subsp. *neglecta* Kümpel, prezrta močvirnica.

Za to podvrsto ozkoustne močvirnice še nimamo sprejetega slovenskega imena. Mi smo jo poimenovali prezrta močvirnica. Na Gorjancih nad krajem Oštrc jo v bukovem gozdu spremljamo že več let (Kosec) (Sl. 8). Višina rastline je 30 do 50 cm, lahko tudi več, stebelni listi so podobni kot pri ozkoustni močvirnici, ozki, suličasti, spodnji tudi jajčasto suličasti ter valoviti na robovih. Ob steblu niso povešeni. Socvetje je bogato ali pa rahlocvetno. Cvetovi se priprti, nekoliko viseči, nekateri so lepo odprti in v njih prevladuje zelenkasta barva. K tej barvi se pridruži nadih rdečkasto vijolične barve, ki je poudarjena predvsem na medeni ustni. Prehod iz hipohila v epihil je ozek. Epihil je srčasto podolgovate oblike, ki v dolžino meri več kot v širino. Konec epihila

je zavihan navzdol, njegova robova sta rahlo valovita. Kljun na stebričku manjka, je zakrnel ali je usahnil. Prašnica "sedi" na vrhu stebrička. Je avtogamna močvirnica. Cveti konec junija in v začetku julija (Sl. 24, 25).



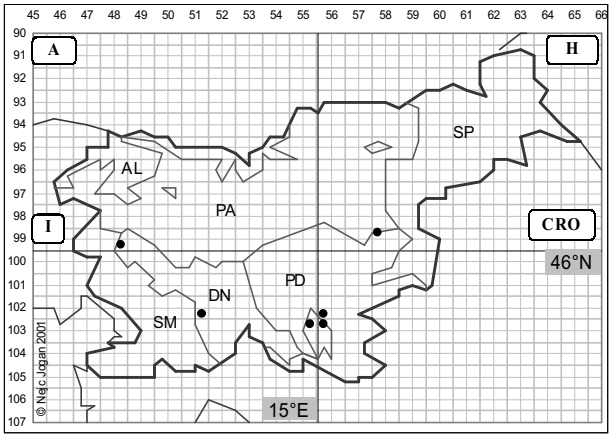
Sl. 8: Fitogeografska razširjenost vrste *E. leptochila* ***subsp.*** *neglecta***.**
Fig. 8: Phytogeographic distribution of the species *E. leptochila* ***subsp.*** *neglecta***.**

Epipactis greuteri H. Baumann & Künkele, greuterjeva močvirnica

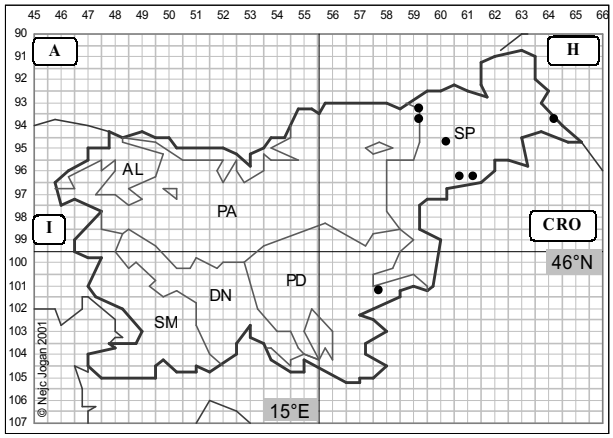
Raste v bukovo-jelovem gozdu na več mestih v Kočevskem Rogu, na Bohorju (Budna, Klenovšek), Javornikih iz Kaliča proti Mašunu (Kosec) in Trnovskem gozdu ob cesti iz Male Lazne proti Lokvam (Kosec) (Sl. 9). Samo od daleč bi jo lahko zamenjali s širokolistno močvirnico. Na prvi pogled daje vtis rahle ovenelosti. Ima dovolj značilnih znakov in je ni težko določiti. Do 80 cm visoka močvirnica ima velike, zelene, suličasto jajčaste liste in gosto, precej enostransko, do polovice stebla segajoče socvetje. Vse na njej daje videz zelenega. Cvetovi so zvonasti in povešeni. Njihovi listi so zašiljeni in rahlo zavihani navzven. Precej dolg pecelj nosi podolgovato, nekoliko ozko plodnico, ta pa zmerno odprt cvet, ki ima znotraj belo ali svetlo rožnato barvo cvetnih listov. Kljun z lepljivo ploščico je viden pri cvetovih samo v zgodnjem obdobju, ali pa ga sploh ni. Če obstaja, kmalu zakrni in kljun ni funkcionalen. Nektar v hipohilu je zelenkaste ali rjave barve, prehod iz hipohila v epihil je zmerno širok. Na belem epihilu najdemo dve majhni zelenkasti izrastlini, ki se na sredini lahko zlijeta. Je avtogamna močvirnica. Cveti konec julija in avgusta (Sl. 26, 27).

Epipactis pontica Taubenh. [*E. helleborine* (L.) Crantz subsp. *Pontica* (Taubenheim*)* Sundermann], pontska močvirnica

Najdemo jo razmeroma pogosto na Štajerskem, v Prekmurju in Dolenjskem (Sl. 10). Verjetno je njeno največje nahajališče v Vinarjih blizu Maribora (Lipovšek), kjer vsako leto v mešanem gozdu zraste 50 primerkov in več. Sodi med manjše močvirnice, saj zraste največ do 40 cm visoko. Stebelni listi so po obliki in razvrstitvi podobni širokolistni močvirnici, so pa manjši. Manjši so tudi cvetovi. Primerjavo smo napravili s fotografiranjem obeh močvirnic hkrati na istem posnetku. Razlike najdemo tudi v zgradbi cveta. Socvetje je gosto do rahlocvetno. Cvetovi so viseči, priprti, prevladuje zelena barva, ki na zunanji strani cvetov kaže rumenkast ton. Notranjost sepalnih listov je zelena, rumenozelenkasta pa pri petalnih listih. Medena ustna ima v zgodnjem obdobja razcveta zeleno barvo nektarja v hipohilu, ki kasneje preide v rjavo in temno rjavo barvo. Prehod iz hipohila v epihil je skledast in širok, vendar se pri



Sl. 9: Fitogeografska razširjenost vrste *E. greuteri***.**
Fig. 9: Phytogeographic distribution of the species *E. greuteri***.**

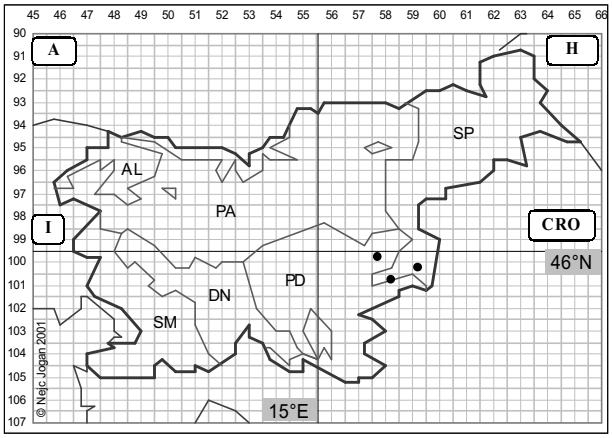


Sl. 10: Fitogeografska razširjenost vrste *E. pontica***.**
Fig. 10: Phytogeographic distribution of the species *E. pontica***.**

začetku epihila prehod nekoliko zoži. Okroglo-rombast epihil je največkrat bele barve z zelenkastima ali rožnato obarvanima majhnima izrastlinama. Konec epihila ni zašiljen. Na stebričku je viden kljun, včasih pa ta manjka. Cveti sredi julija, najbolj v avgustu (Sl. 28, 29).

Epipactis nordeniorum K. Robatsch, nordenova močvirnica

Pri nas je redka močvirnica. Majhno, 10 do največ 30 cm visoko močvirnico težko opazimo, ker se skriva pod više rastočo podrastjo dobovih dreves. V Sloveniji je bila najprej odkrita v Krakovskem gozdu (Kosec), nato še v Dobravi pri Brežicah (Klenovšek) (Sl. 11). Raste predvsem v senci, zelena barva široko jajčastih listov je izrazita. Najdemo jo tudi na robu gozda, takrat pa je močvirnica bolj rožnate barve. Predvsem so takšni njeni cvetovi. Ti pa so pogosto priprti ali zaprti. V odprtih je notranja stran perigonovih listov rumenorožnata, nektar je rjave ali rdečkaste barve, prehod iz hipohila v epihil je izrazito ozek. Epihil je bel ali rožnat, spredaj zavihan navzdol in nazaj ter ima nakazani rožnati izrastlini. Cveti konec meseca julija in v avgustu (Sl. 30, 31).



Sl. 11: Fitogeografska razširjenost vrste** E. norderiorum**.
Fig. 11: Phytogeographic distribution of the species** E. norderiorum**.

Trenutne nedorečenosti okrog taksonov, ki jih spremljamo na terenu

Ozkoustni močvirnici je podobna močvirnica iz Lendave. Našel jo je Paušič že leta 1998. V bukovem gozdu ob Lendavskih goricah so tri nahajališča, kjer vsako leto zraste 20 primerkov in več. Na teh rastiščih je bilo julija 2006 skupaj 41 primerkov (Paušič, Lipovšek). Močvirnica raste v senčnem podrastju bukovja, najdemo pa jo tudi na robu gozda. V višino meri največ 70 cm in ima široke, zelene ovalne liste, ki ob steblu navzgor postajajo ožji, suličasti ter imajo valovit rob. Cvetovi so viseči, lahko priprti, spodnji, v razmeroma bogatem socvetju, so pogosto odprti. Cvet ima podolgovat zašiljen epihil, ki je podoben epihilu ozkoustne močvirnice na Lisci. Stranska robova epihila sta zavihana navzgor, česar pri drugih močvirnicah nismo opazili. Prehod iz hipohila v epihil ima obliko črke V, podobno kot jo ima ozkoustna močvirnica, vendar je širši. Nad epihilom se prehod ne konča v izrazitem žlebiču, kot ga vidimo pri ozkoustni močvirnici. Primerjavo smo napravili s fotografiranjem cveta iz Lisce in Lendave na istem diapozitivu. Zgradba stebrička je podobna ozkoustni močvirnici, kljun hitro zakrni ali pa ga sploh ni. Tudi pogled s strani na položaj prašnice nad stebričkom kaže jasen prehod nad klinandrom. Močvirnica pri Lendavi cveti že v prvih dneh julija na višini okoli 300 mnv. Menimo, da gre za taksonomsko ne dokončno opredeljeno močvirnico, ki je najbolj podobna ozkoustni močvirnici (Sl. 32, 33).

V začetku avgusta leta 2000 smo našli v Kočevskem Rogu ob gozdni cesti razmeroma nizko rastočo, okoli 30 cm visoko močvirnico, ki je imele lepo odprte rdečkasto zelene cvetove. Njihova zgradba je bila podobna, lahko bi celo rekli enaka, cvetovom širokolistne močvirnice. Stebelni listi pa so bili precej drugačni, razmeroma ozki, razvrščeni nasprotno ob steblu in so kazali valovit rob. Šele po večkratnih primerjavah s slikami drugih objav (Presser, 2002) smo zaključili, da gre najverjetneje za *E. helleborine* subsp. *minor*, majhno močvirnico. Nekateri avtorji menijo, da je majhna močvirnica pravzaprav širokolistna močvirnica ali morda le njena različica. Ponovni poskus, da bi jo našli, je obrodil sadove leta 2004 v Dobravi pri Brežicah (Kosec). Na teh rastiščih jo bomo skušali najti vnovič, a do zdaj nam to še ni uspelo (Sl. 34, 35).

Podrobna lokaliteta vseh navedenih močvirnic je na voljo pri avtorjih.

RAZPRAVA

Podobnost med močvirnicami iz kroga širokolistne močvirnice je velika. Zato nam je pri določanju v pomoč slika ali risba. Slednja lahko nazorno pokaže podrobnosti, ki si jih najlaže ogledamo z lupo. To velja predvsem za zgradbo stebrička, kljuna, položaj in kompaktnost polinijev ter obstoj klinandra. Risba je v pomoč tudi pri ogledovanju prehoda iz hipohila v epihil, kar pa terja nekaj terenskih izkušenj, preden lahko rečemo, ali je prehod ozek, širok, skledast, ima obliko črke V itn. Prav te podrobnosti povzročajo veliko težav, kar je razlog, da nekateri avtorji ne ponujajo določevalnega ključa, temveč rajši priporočajo primerjavo močvirnice "na terenu" s fotografijo (ali sliko) posamezne močvirnice. Mnoge sodobne slikovne izdaje o kukavičevkah nam lahko prav zato olajšajo primerjavo in določanje posameznih taksonov. Ne glede na takšen pristop se še vedno lahko znajdemo pred uganko, kam ta ali ona močvirnica sodi, še zlasti, če gre za podobnosti s širokolistno močvirnico.

Ob tem je treba vedeti, da je krog podobnih močvirnic, ki jih tu naštevamo, precej širši, če ga gledamo z evropskega vidika (Ravnik, 1976). Kot primer naj omenimo "nove" močvirnice, ki jih je objavil hrvaški orhidolog Kranjčev (2005), a jih pri nas pod takšnimi imeni ne poznamo. Zanimiv je pri tem podatek, da ne rastejo daleč od naše vzhodne slovenske meje. Med poznavalci in ljubitelji kukavičevk na našem ozemlju so se v preteklosti pojavljala vprašanja, ali ne cveti na Kraškem robu, na Gorjancih in v Istri *E. gracilis*, blizu Senovega *E. albensis,* na Goričkem *E. voethii* itn. Čaka nas torej še precej terenskega dela, če bomo želeli razjasniti številna vprašanja, ki se nam zastavljajo skoraj vsako leto. Ob naštevanju taksonov v tem prispevku ni mišljeno, da bi dokončno opredelili posamezne primerke močvirnic. Že dejstvo, da se nekateri "laboratorijski botaniki" opirajo na genetsko variabilnost (sekvence DNK), kar naj bi pripomoglo predvsem pri določanju filogenije posameznih vrst (Foley & Clarke, 2005), kaže, da bo veliko težav pri usklajevanju morfoloških prvin posameznih taksonov in izsledkov DNK analiz. Vprašanje pa je, ali bodo takšne primerjave sploh možne. Do tedaj pa naj velja klasična razvrstitev, ki je lahko v pomoč predvsem "terenskemu botaniku" in na osnovi katere je bilo ogromno storjenega prav v zadnjih 30 letih.

ZAKLJUČEK

Namen te raziskave je vzpodbuditi pozornost do močvirnic, ki po svoji podobi spominjajo na širokolistno močvirnico in jih je težko določiti. Postopno uvrščanje posameznih močvirnic iz tega kroga lahko spremljamo v zadnjem MFS, v ključu "Naše orhideje" (Jogan, 2000) in v knjigi "Orhideje Slovenije" (Ravnik, 2002). Brez dvoma je problem omenjenih močvirnic večplasten, predvsem kar zadeva njihovo določevanje na terenu. Prav gotovo nas čaka še veliko raziskav, ki bodo verjetno pripeljale do odkritja novih ali podobnih taksonov, ki jih omenjamo v tem prispevku. Korak naprej je morda razdelitev na alogamne in avtogamne vrste, vendar je treba poudariti, da meja med njimi ni vedno jasna. To pa je tudi v skladu z opazovanji drugih avtorjev.

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A CONTRIBUTION TO AN OVERVIEW OF TAXA FROM THE GROUP OF *EPIPACTIS HELLEBORINE* S.L.

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SUMMARY

The article presents an overview of allogamous and autogamous taxa from the group of the Epipactis helleborine *– the Broad-leaved Helleborine. For the flora of Slovenia, two newly discovered taxa are presented:* Epipactis helleborine *subsp.* orbicularis *and* Epipactis leptochila *subsp.* neglecta. *Two further taxa of* Epipactis *have been*

discovered, but their classification remains incomplete. One taxon comes from the region of Lendava, whereas the other has been temporarily assessed as Epipactis helleborine *subsp.* minor. *The determination key to the taxa of the* Epipactis helleborine *group from Slovenia has been generated.*

Key words: *Epipactis*, orchids, survey of taxa, key for determination, Slovenia

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Sl. 12/Fig. 12: *E. helleborine* ***subsp.*** *helleborine* ***(25.7.2004).***
Sl. 13/Fig. 13: *E. helleborine* ***subsp.*** *helleborine* ***(25.7.2004).***
Sl. 14/Fig. 14: *Epipactis helleborine* ***subsp.*** *orbicularis* ***(3.7.2005, Mežica).***
Sl. 15/Fig. 15: *E. helleborine* ***subsp.*** *orbicularis* ***(23.7.2005, Bohor).***
Sl. 16/Fig. 16: *Epipactis latina* ***(26.6.2001, Kraški rob).***
Sl. 17/Fig. 17: *E. Latina* ***(26.6.2001, Kraški rob).***
Sl. 18/Fig. 18: *Epipactis leutei* ***(5.8.2005, Srednji Vrh nad Martuljkom).***
Sl. 19/Fig. 19: *E. leutei* ***(5.8.2005, Srednji Vrh nad Martuljkom).***
Sl. 20/Fig. 20: *Epipactis muelleri* ***(28.6.1998, Kraški rob).***
Sl. 21/Fig. 21: *E. muelleri* ***(28.6.1998, Kraški rob).***
Sl. 22/Fig. 22: *Epipactis leptochila* ***subsp.*** *leptochila* ***(8.7.2006, Lisca).***
Sl. 23/Fig. 23: *E. leptochila* ***subsp.*** *leptochila* ***(8.7.2006, Lisca).***
Sl. 24/Fig. 24: *Epipactis leptochila* ***subsp.*** *neglecta* ***(8.7.2006, Gorjanci).***
Sl. 25/Fig. 25: *E. leptochila* ***subsp.*** *neglecta* ***(8.7.2006, Gorjanci).***
Sl. 26/Fig. 26: *Epipactis greuteri* ***(2.8.2002, Kočevski Rog).***
Sl. 27/Fig. 27: *E. Greuteri* ***(22.7.2005, Bohor).***
Sl. 28/Fig. 28: *Epipactis pontica* ***(17.7.2005, Maribor).***
Sl. 29/Fig. 29: *E. pontica* ***(14.7.2002, Maribor).***
Sl. 30/Fig. 30: *Epipactis norderiorum* ***(2.8.2002, Krakovski gozd).***
Sl. 31/Fig. 31: *E. norderiorum* ***(2.8.2002, Krakovski gozd).***
Sl. 32/Fig. 32: ***Lendavska močvirnica*** */* *Epipactis* ***from Lendava (4.7.2004, Lendava).***
Sl. 33/Fig. 33: ***Lendavska močvirnica*** */* *Epipactis* ***from Lendava (4.7.2004, Lendava).***
Sl. 34/Fig. 34: *Epipactis helleborine* ***subsp.*** *minor* ***(8.8.2000, Kočevski Rog).***
Sl. 35/Fig. 35: *E. helleborine* ***subsp.*** *minor* ***(8.8.2000, Kočevski Rog).***
(Vse fotografije / All photos: M. Lipovšek)

Sl./Fig. 12

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Izvirni znanstveni članek
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GIBALNA AKTIVNOST IN ŠOLSKI DOSEŽKI UČENCEV DRUGEGA TRILETJA

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IZVLEČEK

Glavni namen raziskave je bil ugotoviti povezanost povprečne dnevne gibalne aktivnosti učencev drugega triletja z ocenami pri slovenščini, matematiki, naravoslovju in tehniki oziroma naravoslovju, družbi in športni vzgoji ter splošnim učnim uspehom. Raziskava je bila opravljena na vzorcu 628 otrok, starih od deset do dvanajst let, iz severovzhodne Slovenije. Za ugotavljanje obsega dnevne gibalne aktivnosti, ki dosega vsaj zmerno do visoko stopnjo intenzivnosti, je bil uporabljen vprašalnik, na katerega so odgovarjali učenci. Posamezne predmete in splošni učni uspeh so ocenili učitelji z ocenami od 1 do 5. Razlike v gibalni aktivnosti glede na dosežene ocene so bile izračunane z analizo variance. Rezultati kažejo, da je obseg dnevne gibalne aktivnosti učencev povezan z ocenami večine predmetov in splošnim učnim uspehom ob koncu leta. Ocene slovenščine, matematike, naravoslovja in tehnike oziroma naravoslovja, družbe oziroma geografije in zgodovine ter splošni učni uspeh so v pozitivni zvezi z gibalno aktivnostjo, pri tem so najbolj aktivni odlično ocenjeni učenci. Presenetljivo pa gibalna aktivnost učencev ni pomembno povezana z oceno pri športni vzgoji. Spodbudne so ugotovitve, da učna uspešnost premo sorazmerno narašča z obsegom dnevne gibalne aktivnosti.

Ključne besede: gibalna aktivnost, učni uspeh, povezanost, učenci, drugo triletje

ATTIVITÀ FISICA E RISULTATO SCOLASTICO DI ALUNNI DEL SECONDO TRIENNIO

SINTESI

Lo scopo principale dello studio era quello di accertare la connessione fra l'attività fisica giornaliera media di alunni del secondo triennio e i voti conseguiti in lingua slovena, matematica, scienze naturali ed educazione tecnica (ossia scienze naturali), conoscenza della società, educazione fisica e con il risultato scolastico generale. La ricerca è stata condotta su un campione comprendente 628 bambini, di età fra i dieci e i dodici anni, provenienti dalla Slovenia nord-orientale. Al fine di accertare il grado di attività fisica giornaliera, con intensità da moderata ad alta, agli alunni è stato chiesto di compilare un questionario. I voti alle singole materie ed al risultato scolastico generale, compresi tra 1 e 5, sono stati assegnati dagli insegnanti. Le differenze nell'attività fisica in relazione ai voti conseguiti sono state calcolate con l'analisi della varianza. I risultati indicano che il grado di attività fisica giornaliera degli alunni è correlato ai voti della gran parte delle materie ed al risultato scolastico generale alla fine dell'anno. I voti in lingua slovena, matematica, scienze naturali ed educazione tecnica (ossia scienze naturali), conoscenza della società (ossia geografia e storia) ed il risultato scolastico generale hanno una correlazione positiva con l'attività fisica, e gli alunni con risultati ottimi sono quelli più attivi fisicamente. Stupisce invece che l'attività fisica degli alunni non sia correlata al voto conseguito in educazione fisica. La scoperta che il risultato scolastico cresce in modo proporzionale al grado di attività fisica giornaliera è stimolativo.

Parole chiave: attività fisica, risultati scolastici, connessione, alunni, secondo triennio

UVOD

V zadnjih letih je vse več interesa za preučevanje vpliva, ki ga ima gibalna aktivnost na različne psiho-socialne razsežnosti pri otrocih. Opravljenih je bilo kar nekaj raziskav, v katerih so ugotavljali, kako je gibalna aktivnost povezana z različnimi vidiki kognitivnega delovanja in šolskimi dosežki (npr. Caterino & Polak, 1999; Dexter, 1999; Planinšec, 2002; Planinšec & Pišot, 2003; Tomporowski, 2003; Pišot & Planinšec, 2005; Taras, 2005). Interes za takšne raziskave je posledica vse manjše vključenosti ali celo popolne izključenosti športne vzgoje v šolskih kurikulih nekaterih držav in hkrati izrazite naklonjenosti "kognitivnim" predmetov, z utemeljitvijo, da športna vzgoja po nepotrebnem samo skrajšuje čas, ki bi ga sicer lahko namenili pouku drugih predmetov. Nasprotno pa športni strokovnjaki vedno znova poskušajo dokazati, da redna gibalna aktivnost pomembno vpliva ne samo na telesni in gibalni razvoj otrok, temveč tudi na boljšo kognitivno učinkovitost in posledično boljši učni uspeh (Shephard, 1997). Nekateri celo menijo, da ravno to zagotavlja kredibilnost športni vzgoji kot predmetu v šolskem kurikulu (Kirkendall, 1985).

Novejše študije kažejo, da gibalna aktivnost povzroči v organizmu različne spremembe, ki bi lahko vplivale na boljšo kognitivno učinkovitost in posredno na učni uspeh (Sibley & Etnier, 2003). Kot ozadje povezave med gibalno aktivnostjo in kognicijo so raziskovalci izpostavili kar nekaj pomembnih dejavnikov različnih fizioloških in razvojnih mehanizmov (Tomporowski, 2003). Področja možganov, ki sodelujejo pri gibanju in učenju, so med seboj tesno povezana. Redna gibalna aktivnost lahko te živčne povezave okrepi, hkrati pa vpliva tudi na določene strukturne spremembe centralnega živčevja (Shephard, 1997; Jensen, 1998). Učenje kompleksnih gibalnih spretnosti stimulira prefrontalni korteks, ki je aktiven pri reševanju problemov, kar lahko posledično izboljša učinkovitost učenja. Nadalje, gibalna aktivnost prek različnih mehanizmov dvigne stopnjo splošne vzburjenosti centralnega živčevja, kar vpliva na otrokovo pozornost pri pouku (Shephard, 1997; Sibley & Etnier, 2003). V določenih razmerah naj bi gibalna aktivnost spodbudila kognitivne procese, ki so ključnega pomena za koncentracijo in reševanje problemov (Tomporowski, 2003). Nekatere raziskave dokazujejo, da gibalna aktivnost pospeši krvni obtok v možganih, vpliva na spremembe možganskih nevrotransmiterjev, predvsem povišanje ravni endorfinov, kar naj bi posledično zmanjšalo stres, izboljšalo razpoloženje in povzročilo učinek umirjenosti po vadbi (Fleshner, 2000; Sibley & Etnier, 2003), vse to pa bi lahko imelo pozitivne učinke na kognitivno delovanje in učno uspešnost.

Gibalna aktivnost lahko zagotavlja izkušnje, ki še posebej pri mlajših otrocih spodbudno vplivajo na kognitivni razvoj (Leppo *et al.*, 2000). Otroci, ki v kognitivnem razvoju hitreje napredujejo, lahko dosežejo boljši učni uspeh. Gibalno aktivni učenci so v šoli sposobni ohranjati dobro koncentracijo od prve do pete učne ure, česar pri neaktivnih učencih ni opaziti (Roth, 2005). Redna gibalna aktivnost otrok naj bi izboljšala učno motivacijo, samopodobo, samozavest, šolske dosežke, mentalno pripravljenost, navezovanje stikov, šolsko disciplino ter nenazadnje zdravje, kar pomeni manj manjkajočih dni v šoli. V nasprotju z nekaterimi drugimi avtorji Roth (2005) meni, da gibalna aktivnost izboljša predvsem tisti del učne uspešnosti, ki je, pogojno rečeno, neodvisen od inteligentnosti. Športna vzgoja v šoli učencem zagotavlja tudi socialno interakcijo, učenci se učijo sodelovanja ter spoštovanja določenih pravil, kar jim daje občutek tesnejše povezanosti z razredno skupnostjo in šolo, to pa naj bi spodbudno vplivalo na učni uspeh (Taras, 2005). Gibalno aktivni mladostniki so manj nagnjeni k rizičnim oblikam vedenja, kar naj bi bilo povezano z boljšimi učnimi dosežki (Patel & Luckstead, 2000).

Narejenih je kar nekaj presečnih preglednih raziskav, ki so obravnavale zvezo med gibalno aktivnostjo ter učnimi dosežki osnovnošolcev (npr. Thomas *et al.*, 1994; Pišot & Zurc, 2003). Obstajajo pa tudi vzdolžne študije, v katerih so preučevali vpliv posebej prilagojenih športnih programov na šolske dosežke. Za najtemeljitejše na tem področju veljajo naslednje štiri raziskave: Vanves (povzeto po Shephard, 1997), Trios Riviers Study (Shephard & Lavallee, 1994), The School Health, Academic Performance and Exercise Study (Dwyer *et al.*, 2001) in SPARK (Sallis *et al.*, 1999). Ob teh je le še peščica raziskav, ki so eksperimentalno zastavljene (npr. Klojčnik, 1977; Vauhnik, 1984; Raviv, & Low, 1990; Caterino & Polak, 1999). Na osnovi tako majhnega števila in velikih metodoloških razlik med raziskavami je težko priti do trdnejših zaključkov, kljub temu pa velja poudariti, da pomembnejšega vpliva gibalne aktivnosti na izboljšanje učnih dosežkov večinoma niso dokazali. Kot ključno spoznanje navajajo, da več časa, namenjenega športni vzgoji v šoli, ne vpliva negativno na učne dosežke osnovnošolcev, v določenih segmentih je opazen sicer pozitiven, vendar majhen učinek (Shephard, 1997; Sallis in drugi, 1999; Dwyer *et al.*, 2001). Gibalno aktivnejši učenci so v primerjavi z manj aktivnimi vrstniki dosegali nekoliko višje ocene predvsem pri matematiki in jeziku, v ocenah drugih predmetov pa pomembnih razlik večinoma ni bilo (Shephard & Lavallee, 1994; Dwyer *et al.*, 2001). Tuckman (1999) je na osnovi obsežne analize ugotovil, da ima redna gibalna aktivnost majhen, skoraj zanemarljiv vpliv na otrokovo inteligentnost, kognitivne spretnosti, samospoštovanje, nadzor vedenja ter razpoloženje, ob tem pa navaja, da se pozitivne koristi kažejo predvsem v izboljšanju otrokovih fizioloških funkcij.

Zveza med gibalno aktivnostjo in šolskimi dosežki je v določenih pogledih še vedno nepojasnjena, zato smo na populaciji naših otrok opravili presečno pregledno raziskavo, katere glavni namen je bil ugotoviti, ali je obseg dnevne gibalne aktivnosti povezan z ocenami pri sloven-

ščini, matematiki, naravoslovju in tehniki oziroma naravoslovju, družbi, športni vzgoji in s splošnim učnim uspehom. Na osnovi nekaterih dosedanjih ugotovitev predpostavljamo, da bodo imeli gibalno aktivnejši učenci boljši učni uspeh.

METODE

Vzorec udeležencev. Raziskavo smo napravili na stratificiranem skupinskem slučajnostnem vzorcu otrok. Iz seznama osnovnih šol, ki sodijo v severovzhodni del Slovenije, smo izbrali deset šol. Vzorec je obsegal 628 otrok iz drugega triletja, od tega jih je bilo 252 iz mestnih ter 376 iz izvenmestnih šol, dečkov je bilo 304, deklet 324, stari so bili od 10 do 12 let (AS=11,22; SO=0,80). Starši in otroci so se s sodelovanjem v raziskavi strinjali. Vsi otroci so bili v času zbiranja podatkov zdravi in tudi drugače ni bilo posebnih razlogov, zaradi katerih bi bila njihova običajna gibalna aktivnost omejena.

Gibalna aktivnost. Za ugotavljanje obsega gibalne aktivnosti je bil uporabljen vprašalnik, ki je nastal na osnovi dveh v tujini uveljavljenih vprašalnikov (Crocker *et al.*, 1997; Manios *et al.*, 1998) in je prilagojen glede na nekatere posebnosti pri nas. Vprašalnik je omogočal oceno povprečne dnevne gibalne aktivnosti, ki dosega zmerno do visoko stopnjo intenzivnosti. Otroci so izpolnjevali vprašalnik sedem zaporednih dni. Dosedanje izkušnje so pokazale, da sedemdnevno zaporedno spremljanje zagotavlja dovolj zanesljivo oceno običajnega gibalnega vedenja otrok (npr. Trost *et al.*, 2000). Vprašalniki sicer veljajo za nizko do srednje zanesljive pri oceni gibalne aktivnosti in so primerni predvsem za večje vzorce merjencev in epidemiološke raziskave, pri katerih so druge metode skoraj neuporabne (Planinšec, 2003).

Šolski dosežki. V raziskavi so bile upoštevane številčne ocene od 1 do 5, ki so jih učenci dosegli ob koncu šolskega leta pri slovenščini, matematiki, družbi (učenci 4. in 5. razreda) oz. geografiji in zgodovini (učenci 6. razreda), športni vzgoji, naravoslovju in tehniki (učenci 4. in 5. razreda) oziroma naravoslovju (učenci 6. razreda) in splošni učni uspeh. Zavedamo se, da učitelji pri ocenjevanju zagotovo niso uporabili povsem enakih kriterijev, vendar se subjektivnosti pri ocenjevanju ne da povsem izogniti; z enakim problemom se srečujejo tudi v drugih raziskavah (npr. Dwyer *et al.*, 2001). O posebnih težavah pri ocenjevanju učitelji niso poročali.

Statistična obdelava podatkov. Zbrane podatke smo analizirali s statističnimi metodami, ki so primerne za analizo podatkov v presečnih preglednih raziskavah. Izračunali smo osnovne statistične kazalce. Razlike v gibalni aktivnosti glede na ocene posameznih predmetov in splošni učni uspeh smo izračunali z analizo variance (splošni F-preizkus) s preizkusom homogenosti populacijskih varianc (Levene-preizkus). Uporabljen je bil Tukeyev post hoc-preizkus. Rezultate smo vrednotili kot statistično pomembne pri vrednosti $p<0,05$. Obdelava podatkov je bila opravljena s programom SPSS 12.0.1.

REZULTATI

V Tabeli 1 so prikazani izidi analize variance razlik v gibalni aktivnosti glede na splošni učni uspeh ter ocene slovenščine, matematike, naravoslovja in tehnike/naravoslovja, družbe in športne vzgoje. Kakor kaže izid F-preizkusa učinka faktorja splošni učni uspeh ($F = 11,777$; $p = 0,001$), je razlika med učenci v gibalni aktivnosti statistično pomembna, pri tem so najmanj aktivni učenci, ki so bili ob koncu leta zadostni (52,92 min/dan), največ pa odlični (83,20 min/dan). Dobri in prav dobri učenci so bili aktivni nekaj več kot 69 minut dnevno. Tukeyev post hoc-preizkus je pokazal, da je razlika, razen med dobrimi in prav dobrimi, v vseh drugih primerih statistično pomembna. Izid analize variance razlik v gibalni aktivnosti glede na ocene pri slovenščini kaže, da obstaja med učenci pomembna razlika ($F=11,347$; $p=0,001$). Najbolj aktivni so odlično ocenjeni učenci (80,47 min/dan), sledijo prav dobro ocenjeni (77,43 min/dan), dobro in zadostno ocenjeni pa so aktivni nekaj več kot 60 min/dan. Pomembne razlike so med učenci z zadostno oceno ter prav dobro in odlično oceno, pa tudi med dobro ocenjenimi ter prav dobro in odlično ocenjenimi. F-preizkus učinka ocene matematike ($F=10,807$; $p=0,001$) kaže, da

Tab. 1: Povzetek izidov analize variance razlik v gibalni aktivnosti (min/dan) glede na učni uspeh.
Tab. 1: Summary of the results obtained with the analysis of variance of differences in physical activities (min/day) in view of the pupils' school achievements.

Predmet	Ocena	No.	Aritmetična sredina (min/dan)	Standardni odklon	Preizkus razlik AS	
					F	p
Splošni učni uspeh	2	52	52,92	32,94	11,777	0,001
	3	120	69,64	37,89		
	4	224	69,94	37,94		
	5	232	83,20	36,56		
Slovenščina	2	64	60,38	33,33	11,347	0,001
	3	124	60,27	34,26		
	4	220	77,43	39,74		
	5	220	80,47	36,84		
Matematika	2	60	64,25	40,00	10,807	0,001
	3	112	60,54	36,22		
	4	248	72,91	36,95		
	5	208	83,46	36,94		
Naravoslovje in tehnika / Naravoslovje	2	36	62,82	40,71	8,734	0,001
	3	108	69,64	39,80		
	4	224	66,55	38,76		
	5	260	82,26	34,34		
Družba/ geografija in zgodovina	2	56	61,33	35,48	5,476	0,001
	3	136	65,72	40,65		
	4	176	76,17	36,10		
	5	260	78,07	37,32		
Športna vzgoja	2	8	90,86	21,52	0,765	0,514
	3	20	70,52	37,87		
	4	132	71,21	38,19		
	5	468	73,80	38,14		

je razlika med učenci v gibalni aktivnosti pomembna. Ponovno so najaktivnejši odlično ocenjeni učenci (83,46 min/dan), sledijo prav dobri (72,91 min/dan), precej manj so aktivni zadostni (64,25 min/dan) in dobri (60,54 min/dan). Post hoc-preizkus je pokazal, da je razlika, razen med zadostno in dobro ocenjenimi ter zadostno in prav dobro ocenjenimi, statistično pomembna.

Razlike v gibalni aktivnosti glede na oceno naravoslovja in tehnike oziroma naravoslovja so statistično pomembne (F=8,734; p=0,001). Predvsem se kažejo pri odlično ocenjenih učencih, ki so aktivni 82,26 min/dan, vsi drugi so aktivni mnogo manj. Tukeyev post hoc-preizkus je pokazal, da je razlika pomembna samo med učenci z odlično oceno in vsemi drugimi, sicer pa ni pomembna. Izid F-preizkusa učinka faktorja družba oz. geografija in zgodovina (F = 5,476; p = 0,001) kaže, da je razlika med učenci v gibalni aktivnosti statistično pomembna, pri tem so najbolj aktivni odlično ocenjeni (78,07 min/dan), najmanj pa učenci z zadostno oceno (61,33 min/dan). Post hoc-preizkus je pokazal, da obstaja pomembna razlika med učenci z odlično in prav dobro oceno na eni strani ter dobro in zadostno oceno na drugi strani. Zelo presenetljivi so izidi splošnega F-preizkusa učinka faktorja športna vzgoja, saj razlike v gibalni aktivnosti med različno ocenjenimi učenci niso statistično pomembne (F=0,765; p=0,514), kljub temu pa velja poudariti, da so najaktivnejši učenci, ki so pri športni vzgoji ocenjeni zadostno (90,86 min/dan).

RAZPRAVA

Najpomembnejše spoznanje raziskave je, da je obseg povprečne dnevne gibalne aktivnosti učencev povezan z njihovimi šolskimi dosežki, kar so ugotavljali tudi v drugih raziskavah (npr. Thomas *et al.*, 1994; Pišot & Zurc, 2003). Po pričakovanju je gibalna aktivnost premo sorazmerna s splošnim učnim uspehom in ocenami pri posameznih predmetih. Najaktivnejši so bili učenci, ki so dosegli odličen učni uspeh, najmanj aktivni pa so bili učenci z zadostnim uspehom. Podobno velja tudi za ocene slovenščine, matematike, naravoslovja in tehnike oz. naravoslovja ter družbe oz. geografije in zgodovine.

Zanimivo pa je, da obseg gibalne aktivnosti in ocena pri športni vzgoji nista pomembno povezana. To je v nasprotju s pričakovanji in z dosedanjimi spoznanji, kjer je ugotovljena skromna, vendar pozitivna zveza med gibalno aktivnostjo in oceno športne vzgoje (Dexter, 1999). Če kje, potem bi predvsem pri oceni športne vzgoje lahko nastale razlike glede na gibalno aktivnost. Pričakovali smo, da bodo imeli aktivnejši učenci več športnega znanja in bodo pri športni vzgoji bolje ocenjeni, vendar rezultati teh predvidevanj niso potrdili. Najpomembnejši razlog za takšno stanje je verjetno metodološke narave, saj je velika večina učencev, kar 75%, pri športni vzgoji odlično ocenjenih, 21% pa prav dobro. Zato je primerjava z dobro in zadostno ocenjenimi, ki jih je skupaj le 4%, praktično nesmiselna. Pri drugih predmetih je delež odlično ocenjenih bistveno manjši in se giblje med 33% (matematika) in 41% (naravoslovje in tehnika, družba). Nasprotno pa je z deležem zadostno ocenjenih, ki jih je pri športni vzgoji 1,3%, torej mnogo manj kot pri drugih predmetih (od 5,7% pri naravoslovju in tehniki do 10% pri slovenščini). Krivulja porazdelitve ocen pri športni vzgoji je bistveno drugačna kot pri drugih predmetih in splošnem uspehu. To kaže, da je v primerjavi z drugimi predmeti športno znanje učencev očitno mnogo boljše in tudi bolj izenačeno. Morda pa zelo visok delež odlično in prav dobro ocenjenih kaže tudi na bolj pozitivno naravnanost učiteljev pri ocenjevanju športne vzgoje oziroma na manj strog kriterij ocenjevanja glede na druge predmete.

Z dosedanjimi raziskavami je skladna ugotovitev, da je gibalna aktivnost pozitivno povezana z uspehom pri matematiki (Shephard & Lavallee, 1994; Thomas *et al.*, 1994; Dexter, 1999; Dwyer *et al.*, 2001); nasprotno pa Sallis *et al.*, (1999) takšne povezave niso dokazali. Razlike med študijami so verjetno posledica metodologije ocenjevanja pri matematiki. Samo v študiji SPARK (Sallis *et al.*, 1999) so pri ocenjevanju matematike uporabljali standardizirane teste, v drugih primerih pa so, podobno kot v naši študiji, učitelji podali oceno po lastni presoji, kar je verjetno povzročilo določene odmike v kriterijih ocenjevanja.

Skladnost spoznanj naše raziskave z dosedanjimi raziskavami je tudi pri povezavah gibalne aktivnosti in jezikovnih predmetov, čeprav so dokazi večinoma nepreprič̌ljivi (Shephard & Lavallee, 1994; Dexter, 1999; Sallis *et al.*, 1999; Dwyer *et al.*, 2001), podobo je tudi, ko gre za splošni učni uspeh (Sallis *et al.*, 1999).

Ključno vlogo pri povezavah gibalne aktivnosti in šolskih dosežkov imajo verjetno socialno-kulturni dejavniki. Predvsem bi veljalo poudariti socialno-ekonomski položaj družine ter izobrazbo staršev, ki pa jih v raziskavi nismo kontrolirali. Strel *et al.* (2005) ugotavljajo, da so gibalno aktivnejši otroci vključeni v več različnih "nešportnih" interesnih dejavnosti kot njihovi manj aktivni vrstniki, kar lahko vpliva na boljši učni uspeh. Razlog je morda tudi stopnja izobrazbe staršev, ki še vedno sodi med dejavnike, ki so premo sorazmerno povezani z učnim uspehom osnovnošolcev (Peček *et al.*, 2006). Poleg tega so višje izobraženi odrasli bolj gibalno aktivni od manj izobraženih (Sila & Doupona Topič, 2001). Moore *et al.* (1991) so ugotovili, da je verjetnost, da bodo otroci gibalno aktivni, 5,8-krat večja, če so aktivni tudi starši. Na osnovi teh navedb bi lahko sklepali, da so v družinah bolj izobraženih staršev tudi otroci bolj gibalno aktivni. Pišot & Zurc (2003) predpostavljata, da sta gibalna aktivnost in učni uspeh povezana zato, ker imajo učno uspešnejši učenci morda več prostega časa in so lahko bolj aktivni.

Pričujoča raziskava ni bila zasnovana tako, da bi lahko ugotavljali vpliv gibalne aktivnosti na učne dosež-

ke. V ta namen bi morali napraviti vzdolžno raziskavo, ki pa bi bila z izvedbenega in finančnega vidika izjemno zahtevna. Poleg tega dosedanja spoznanja o vplivu gibalne aktivnosti na kognitivno delovanje in učni uspeh niso ravno spodbudna (Taras, 2005), zato je vprašanje, ali bi bila takšna raziskava sploh smiselna. Horga (1993) navaja, da zveza med gibalno aktivnostjo in kognitivno učinkovitostjo sicer obstaja, vendar ni dvosmerna, ampak večinoma le enosmerna, in to v smislu pozitivnega vpliva kognitivnih sposobnosti na gibalno učinkovitost oziroma uspeh v športu, v nasprotni smeri pa naj bi bil vpliv nevtralen. Tega ne potrjujejo samo znanstvena spoznanja, temveč tudi narava kognitivnih sposobnosti, njihov razvoj ter možnost drugih vplivov na njihov razvoj. Če želimo doseči napredek na kognitivnem področju, je najboljša možnost, kot pravi Horga (1993), da uporabimo športno logiko, po kateri je najboljša oblika vadbe situacijska vadba, kar pomeni, da bi za izboljšanje kognitivne učinkovitosti in učnega uspeha uporabili predvsem različne oblike kognitivnih aktivnosti, ki so jih otroci deležni pri drugih šolskih predmetih. Argumenti, da ukvarjanje s športom pomembno vpliva na kognitivne funkcije in posredno na boljši učni uspeh, žal znanstveno niso dovolj trdno podprti. Najuglednejši strokovnjaki opozarjajo, da je bistveno bolj tehtno in strokovno korektno kot ključne argumente za redno gibalno aktivnost otrok poudariti predvsem vpliv, ki ga ima na zdravje, dobro počutje, telesno pripravljenost, nekatere čustveno-socialne razsežnosti, oblikovanje zdravih navad ter spodbujanje razvoja (Shephard, 1997; Sallis *et al.*, 1999; Dwyer *et al.*, 2001; Taras, 2005).

Zavedamo se omejenosti, ki jih ima naša raziskava in se nanašajo predvsem na metode zbiranja podatkov. Kot smo že omenili, so vprašalniki nizko do zmerno zanesljivi v oceni povprečne dnevne gibalne aktivnosti. Vendar pa pri velikih vzorcih druge metode praktično niso uporabne. Poseben problem so tudi kriteriji ocenjevanja šolskih dosežkov oziroma razlike, ki se pojavljajo med učitelji, čeprav se s tem srečujejo skoraj v vseh študijah (npr. Dwyer *et al.*, 2001). Kljub temu pa zaradi velikega števila razredov in učiteljev, ki so podali oceno otrokove učne uspešnosti, o kakšnem enostranskem, sistematičnem vplivu ne bi mogli govoriti.

Spodbudno je, da smo tudi v naši raziskavi potrdili dosedanja spoznanja (npr. Shephard, 1997; Sallis *et al.*, 1999; Dwyer *et al.*, 2001), da učenci, ki so bolj gibalno aktivni, nimajo slabšega učnega uspeha. Ugotovili smo, da učna uspešnost premo sorazmerno narašča z obsegom dnevne gibalne aktivnosti. Zaradi številnih koristi bi bilo smiselno povečati obseg gibalnih aktivnosti v šolah in vsem učencem vsakodnevno ponuditi kakovostne in zanimive športne programe.

PHYSICAL ACTIVITY AND ACADEMIC ACHIEVEMENTS IN ELEMENTARY SCHOOL CHILDREN (SECOND CYCLE)

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SUMMARY

The present study examined the connections between pupil's reported levels of daily physical activity with the school grades in Slovene, Mathematics, Natural Science and Techniques or Natural Science, Social Science and Physical Education and pupil's general school achievement. The study included 628 children (age 9 to 12 years) from the north-eastern part of Slovenia. A self-reported questionnaire for the assessment of daily physical activity was used. Teachers assessed individual subjects and general school achievement by grades from 1 to 5. Differences in physical activity, regarding the achieved grades, were calculated with the one-way analysis of variance. The results indicate that the level of pupils' daily physical activity is connected with the grades of different subjects and with the general school achievement at the end of the year. Grades in Slovene, Mathematics, Natural Science and Techniques or Natural Science, Social Science or Geography and History and general school achievement are in positive relation to physical activity; the most active are pupils with best grades. Physical activity is, surprisingly, not significantly related to the grades in Physical Education. We have established that academic achievement straight-proportionally increases with the level of daily physical activity.

Key words: physical activity, academic achievement, relations, pupils, elementary education (second cycle)

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KRASOSLOVNE RAZISKAVE PRI GRADITVI AVTOCEST PREK SLOVENSKEGA KRASA

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IZVLEČEK

Krasoslovci sodelujemo pri graditvi avtocest na krasu. S predhodnimi študijami z načrtovalci izbiramo trase, z rednim krasoslovnim nadzorom ob graditvi ceste pa preučujemo na novo odkrite kraške pojave, graditeljem pomagamo premostiti kraške značilnosti in jih hkrati čimveč ohraniti. Pri graditvi najnovejšega dela avtocest se je na 60 km trase odprlo več kot 350 jam. Različne vrste jam odsevajo razvoj pretočno-odtočnega vodonosnika zaradi nižanja gladine podzemeljske vode in kraškega površja.

Ključne besede: gradnja avtocest, Kras, kraška jama, klasični kras, Slovenija

RICERCHE CARSOLOGICHE DURANTE LA COSTRUZIONE DI AUTOSTRADE CHE ATTRAVERSANO IL CARSO SLOVENO

SINTESI

Un gruppo di carsologi collabora alla costruzione di autostrade sul Carso. Con studi preliminari effettuati assieme ai progettisti vengono selezionati i percorsi, mentre con un controllo carsologico costante durante la costruzione autostradale vengono studiati nuovi fenomeni carsici. Il gruppo di studiosi aiuta i costruttori a superare gli ostacoli dovuti alle caratteristiche carsiche e collabora nella salvaguardia di tali peculiarità. Durante la costruzione della parte autostradale più recente, su 60 km di percorso sono emerse più di 350 grotte. Diversi tipi di grotte riflettono l'evoluzione di flussi acquiferi trapassanti ed eflussi, dovuta all'abbassamento del livello dell'acqua sotterranea e della superficie carsica.

Parole chiave: costruzione di autostrade, Carso, grotta carsica, carso classico, Slovenia

UVOD

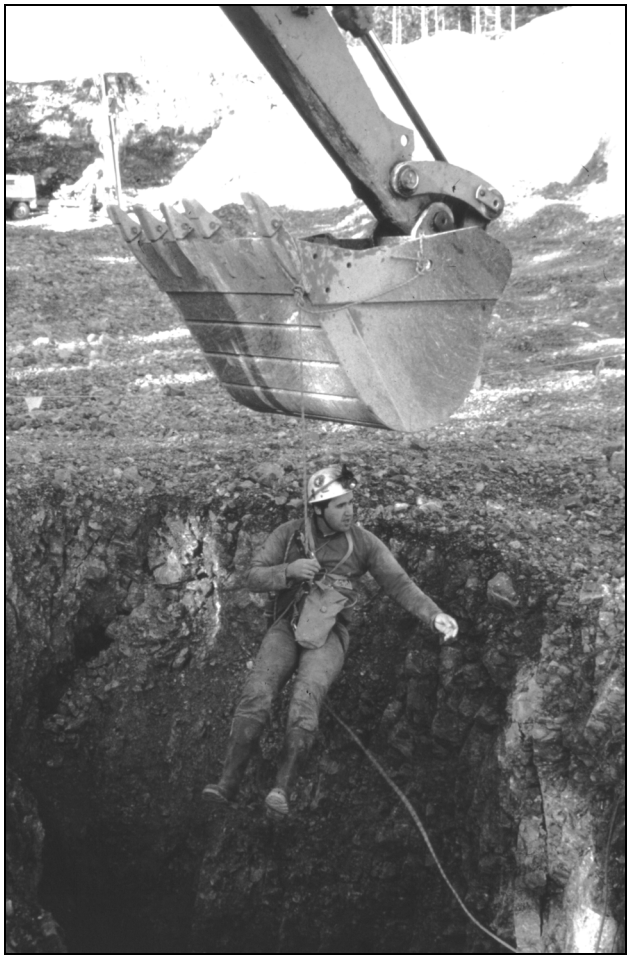
Eden večjih projektov, ki potekajo v Sloveniji, je povezati državo s sodobnimi avtocestami. Skoraj polovica Slovenije je kraške in več kot polovica voda, s katerimi se oskrbujemo, je iz kraških vodonosnikov. Slovenija je dežela klasičnega Krasa, ki je dal ime za to svojevrstno pokrajino na karbonatnih kamninah številnim jezikom sveta in se je na njem začelo razvijati krasoslovje. Občutljiva kraška pokrajina torej terja od nas njeno dobro poznavanje in trud za njeno ohranjanje, hkrati pa je seveda pomemben del naše naravne in kulturne dediščine.

Krasoslovci že vrsto let sodelujemo pri načrtovanju in graditvi avtocest na krasu (Kogovšek, 1993, 1995; Knez *et al.*, 1994; Knez & Šebela, 1994; Šebela & Mihevc, 1995; Slabe, 1996, 1997a, 1997b, 1998; Mihevc, 1996, 1999; Mihevc & Zupan Hajna, 1996; Kogovšek *et al.*,1997; Mihevc *et al.*,1998; Šebela *et al.*,1999; Bosak *et al.*, 2000; Knez & Slabe, 2000, 2001, 2002, 2004a, 2004b, 2005; Knez *et al.*, 2003, 2004). Pri izbiri trase avtocest in železniških prog so v ospredju upoštevanje celovitosti kraške pokrajine, priporočila po izogibanju pomembnejšim površinskim kraškim pojavom (vrtače, polja, udornice, kraške stene) in že znanim jamam. Posebno pozornost posvečamo vplivu graditve in uporabe avtocest na kraške vode. Avtoceste naj bi zato bile neprepustne, vode s cestišča se namreč najprej zberejo v lovilcih olj in so nato prečiščene spuščene v kras.

Preučevali smo vplive prometnic na kraško vodo. Kogovšek (1993, 1995) je ugotavljala sestavo onesnaženosti voda, ki se vsakodnevno stekajo z avtocest. V stoječih vodah, katerih manjše količine smo našli v jamah ob prometnicah, so bile tudi sledi mineralnih olj (Knez *et al.*, 1994).

Med graditvijo avtocest pa opravljamo krasoslovni nadzor. Preučujemo na novo odkrite kraške pojave kot pomemben del naše naravne dediščine, svetujemo način njihove ohranitve, če je to zaradi gradbenih del le mogoče, naša nova spoznanja pa so tudi v pomoč graditeljem (Sl. 1). Pridobili smo vrsto novih izsledkov o oblikovanju in razvoju kraškega površja, epikrasa in prevotljenosti vodonosnika. Na 60 kilometrih avtocest, ki so bile v zadnjih letih zgrajene na Krasu, je bilo na novo odkritih več kot 350 jam.

V prispevku predstavljava naše večletne izkušnje preučevanja kraških pojavov ob graditvi cest, kjer sodelujemo pri načrtovanju in nadzoru gradnje ter dodajava najnovejša spoznanja. Sodiva, da nekaterim sicer znanim kraškim pojavom posvečamo premalo pozornosti. Osredotočila sva se na primere s klasičnega Krasa, krasa, ki je dal ime za pokrajine na karbonatnih kamninah in na katerem se je začelo razvijati krasoslovje.



Sl. 1: Raziskave jame, katere strop se je udrl zaradi miniranja med graditvijo avtoceste pri Divači.
Fig. 1: Exploration of the cave whose roof collapsed due to blasting during the motorway construction near Divača.

NAČRTOVANJE

Pri načrtovanju cest s krasoslovnega vidika ovrednotimo kraško površje, kraško podzemlje, hidrološke posebnosti in ocenimo tudi predstavljene variante. Kjerkoli na krasu, kjer gradimo ceste, naletimo na številne kraške pojave: vrtače (Sl. 2), zapolnjene ali prazne votline ter segmente starih ali recentnih drenažnih poti skozi kras (Sl. 3). Številne kraške jame pa je denudacija že razgalila in jih lahko prepoznamo na površju krasa. V zadnjem času je jamam brez stropa, ki so bile "odkrite" prav med graditvijo avtocest, posvečena posebna pozornost. Zavedamo se, da kvalitetna krasoslovna študija področja, na katerem se načrtuje prometnica, omogoča dober izbor trase in je eno izmed temeljnih izhodišč za načrtovanje graditve v svojevrstni in občutljivi pokrajini.

V prvem koraku s pomočjo objavljene literature, arhivov in različnih zbirk zberemo znanje o površinskih

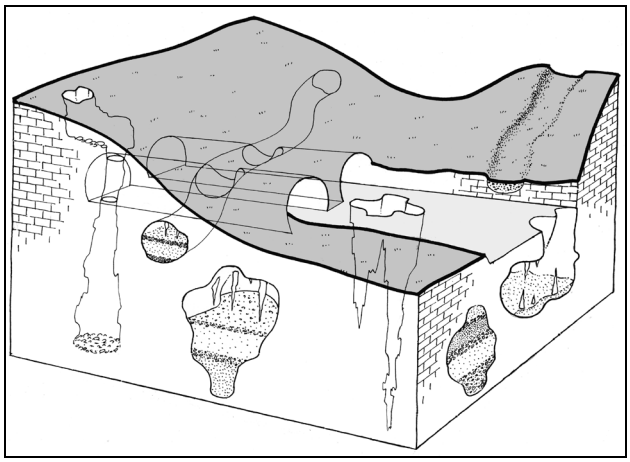
Sl. 2: Zapolnjevanje vrtače z gruščem. Iz vrtače so najprej odstranili naplavine. Danes je pod avtocesto med Kozino in Divačo.
Fig. 2: A doline filled up by rubble after the sediments were removed. Today it lies under the motorway between Kozina and Divača.

kraških pojavih, med katerimi še posebej izločimo doline, vrtače, udore ter druge morfološke oblike. Kasneje s pomočjo terenskega ogleda določimo kriterije za kartiranje območja izbrane trase. Na terenu s krasoslovnega vidika ovrednotimo različne kamninske segmente. Na kartah tematsko predstavimo znane vhode v podzemske prostore ter jih dopolnimo z morebitnimi novimi. Na podlagi površinskega kartiranja in genetske interpretacije morfološko izraženih in v reliefu zaznavnih denudiranih jam napravimo prognozo podzemeljskih votlin. Če je potrebno, na podlagi površinskega kartiranja predvidimo možnost deponij viškov materiala.

Iz izkušenj vemo, da v sleherni trasi, ki prečka kras, med graditvijo naletimo na podzemne votline in dele jamskih sistemov. Obliko in tip votlin lahko delno predvidimo s pomočjo interpolacije površinskih in podzemskih pojavov. Jamam, ki jih zasledimo v širši okolici trase, določimo vrsto, njihov položaj in vlogo v vodonosniku, obliko, skalni relief, naplavine in sigo v njih ter jih predstavimo na ustreznih kartah. Terensko preverimo verodostojnost znanih podatkov ter jih dopolnimo z morebitnimi novimi meritvami ter genetsko interpretacijo (npr. zapolnitve z alohtonimi klastičnimi sedimenti). Zaradi boljšega razumevanja predstavimo dosedanje poznavanje prevotljenosti vodonosnika in izdelamo prognoze s posebnim poudarkom na pričakovanih lito-tektonskih spremembah kamnine.

Zaradi specifičnih lastnosti karbonatne kamnine lahko kraške vode, ki poniknejo na obravnavanem območju, brez težav najdejo neposredne poti v podzemlje (kraški vodonosnik); 100 m debele kamnine lahko preidejo že v dobri uri. Kljub temu da so flišne kamnine, ki so na Krasu v stalnem neposrednem kontaktu s karbonati, pogosto predstavljene kot izključno neprepustni skladi, moramo poudariti, da je fliš (marsikje manjših debelin) le izolirana leča na prepustnih karbonatnih kamninah. Poleg tega je treba tudi vedeti, da se v flišnih kamninah prav tako oblikujejo, sicer manj številni, podzemni prevodni kanali ter da na flišu zbrana padavinska voda odteka v kras. Zato opravimo terensko hidrogeološko kartiranje. V ta namen razmejimo in določimo osnovne značilnosti hidrogeoloških enot na širšem območju trase, popišemo hidrološke objekte (zajeti in nezajeti izviri, površinski tokovi, vodne jame, vrtine, merilne postaje in drugo) ter določimo fizikalno-kemične lastnosti izvirov. Če je treba, opravimo sledilna poizkusa ob nizkih in visokih vodah predvsem za določitev smeri in hitrosti podzemnega toka na širšem območju trase. Izdelamo in nadgradimo obstoječe hidrogeološke karte z rezultati terenskega kartiranja in sledilnih poizkusov, izdelamo inventar o stanju okolja ter opravimo oceno vpliva gradnje na kraške vode.

Kratko lahko strneva temeljne smernice načrtovanja prometnic:
– izbor trase temelji na podlagi celostne presoje krasa s poudarkom na lokalnih značilnostih;
– izbrani potek trase se izogiba tudi posameznim izjemnim kraškim pojavom;
– eden prednostnih ciljev načrtovanja je ohranjanje kraškega vodonosnika.



Sl. 3: Različne jame, ki so se odprle med graditvijo avtoceste (Knez & Slabe, 2005).
Fig. 3: Different caves that opened during the motorway construction (Knez & Slabe, 2005).

KRASOSLOVNI NADZOR OB GRADITVI

Odstranitev prsti in rastja s kraškega površja in seveda večja zemeljska dela pri kopanju cestnih usekov in predorov so razkrila površinske, epikraške in podzemeljske kraške pojave. Naša naloga je te pojave preučiti kot del naravne dediščine, svetovati način njihovega ohranjevanja in seveda seznanjati graditelje z novimi

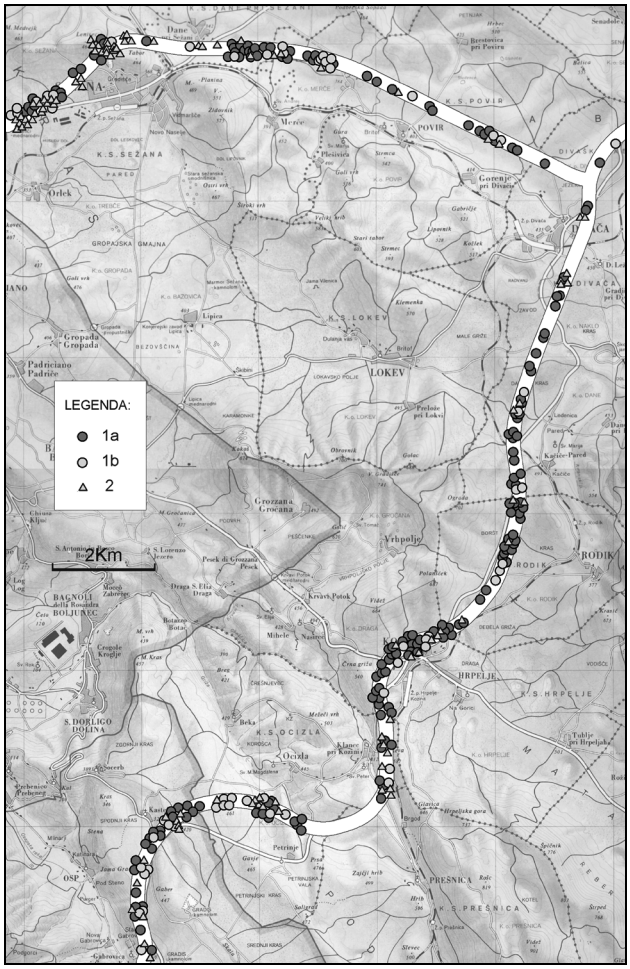
Sl. 4: Jama brez stropa pri Povirju, iz katere so bile odstranjene naplavine in siga.
Fig. 4: A roofless cave near Povir, from which sediments and flowstone were removed.

spoznanji. Ti izsledki jim pomagajo pri premoščanju gradbenih ovir.

Kraško površje členijo vrtače in jame brez stropa (Sl. 4). Vrtače so znamenje današnjega oblikovanja površja s padavinsko vodo, ki navpično prenika skozenj in nato po nezalitem delu vodonosnika do podzemeljskih voda. Nekatere vrtače so bolj, druge pa manj izrazito zapolnjene s prstjo. Na njihovem dnu se odpirajo brezna in špranje, skozi katere odteka voda. Prst je treba odstraniti iz vrtač, dna utrditi s svodasto zloženimi skalami, ustja brezen so namreč pogosto manjša kot bližnje votline pod njimi, in vrtače nato zapolniti s plastmi grušča. Podobnih oblik ali pa bolj podolgovate so jame brez stropa. To so stare jame, ki so spričo znižanja kraškega površja "pogledale" na dan, torej nimajo več zgornjih delov oboda. Tudi iz njih je treba odstraniti drobnozrnate zapolnitve, v tem primeru so to stare jamske naplavine in jame nato zapolniti s skalami in gruščem. Voda bi namreč lahko te naplavine sčasoma odnesla in na površju bi se lahko pojavil grez.

Epikras prepredajo špranje, zlasti izrazite so v krednem apnencu, manj pa v paleogenskem, več se jih je odprlo na dnu in pobočjih vrtač. Večinoma so zapolnjene s prstjo in njihove stene razčlenjene s podtalnimi skalnimi oblikami. Zaradi znižanja kraškega površja je veliko brezen že tik pod njim.

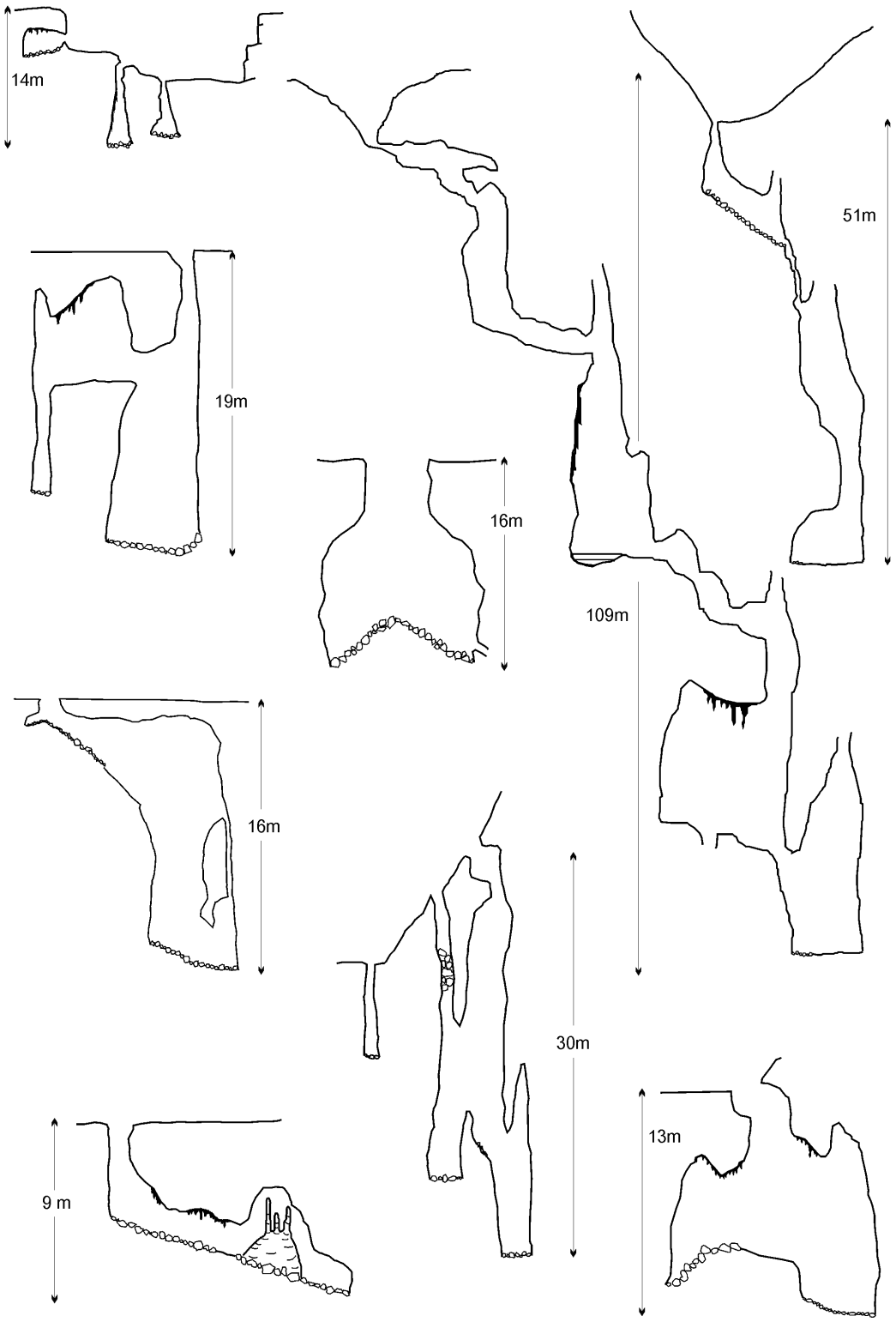
Na 60 km trase avtocest, ki so bile izgrajene v zadnjih letih na Krasu, se je odprlo več kot 350 jam (Sl. 5). Jame lahko glede na razvoj vodonosnika delimo na stare jame, skozi katere so se pretakali vodni tokovi, ko je bil kraški vodonosnik višje obdan in prekrit s flišem in brezna, skozi katere se voda navpično pretaka s prepustnega kraškega površja do podzemeljskih voda. Najgloblje brezno je merilo 110 m (Sl. 6). Stare jame so prazne (Sl. 7) ali pa zapolnjene z naplavino (Sl. 8), slednjih je skoraj dve tretjini jam, ena tretjina jam pa je že brez stropa.



Sl. 5: Jame, ki so se odprle med graditvijo med Razdrtim, Fernetiči in Črnim kalom. Legenda: 1. stare jame: a) jame, zapolnjene s sedimenti in sigo, b) nezapolnjene, votle jame; 2. brezna. Avtocesta je na skici 15-krat širša.
Fig. 5: Caves that opened during the construction in the area from Razdrto, Fernetiči and Črni kal. Legend: 1. old caves: a) caves filled with sediment and flowstone, b) unfilled, empty caves; 2. shafts. Motorway on sketch is 15-times enlarged.

Jame se odpirajo pri odstranjevanju rastja in prsti s površja in še zlasti veliko se jih je odprlo pri kopanju cestnih usekov. Pri miniranju kamnine so se vdrli njihovi stropi, v brežinah pa so se ohranili prečni preseki rovov. Največ brezen se je odprlo na dnu vrtač, ko so odstranili prsti in naplavine iz njih.

Vse jame smo dodobra preučili, narisali načrte, opredelili njihovo obliko, skalni relief, zbrali smo vzorce naplavin za paleomagnetne in pelodne raziskave, vzorce sig pa za mineraloške raziskave in datacije. Na podlagi oblike jame in geoloških danosti smo predvideli njihova nadaljevanja, kar bo še zlasti koristilo graditeljem



Sl. 6: Med graditvijo so se odprle jame različnih oblik in velikosti, kar je narekovalo tudi nadaljnja gradbena dela.
Fig. 6: During the construction, many caves of different shape and size open; this dictated further appropriate construction works.

pri njihovi sanaciji. Skušali smo ohraniti čim več jam. Najlaže smo ohranjali brezna. Njihove manjše vhode smo zaprli z betonskimi ploščami. Prav tako je bilo moč ohraniti stare jame, katerih obodi so bili trdni. Jame, ki pa so se odprle zaradi miniranja in so bile v pretrti kamnini, je bilo treba razminirati in zasuti. Jame, ki so jih presekali useki in katerih vhodi so v njihovih brežinah, pa smo zaprli s skalnatimi zidovi. Njihovi obodi so namreč preveč pretrti in jame so zato neprimerne za nadaljnje obiskovanje, iz jam, ki so zapolnjene z naplavinami, pa bi voda lahko na cestišče odnašala ilovico. Eno izmed dobro ohranjenih jam smo pustili odprto za ogled potnikom, ki prestopajo mejo z Italijo. Najbolj zanimive in dobro ohranjene jame pa smo zaščitili v celoti in čeprav so pod avtocesto, so dostopne. Do njih namreč vodijo betonske cevi, ki se ob cesti zaključijo z zaprtim jaškom.

Preučevali smo tudi posledice različnih miniranj v jamah, kar nam koristi pri nadaljnji graditvi in ohranjanju kraških pojavov.

NOVA SPOZNANJA O RAZVOJU KRASA, PRIDOBLJENA MED GRADITIVIJO AVTOCEST

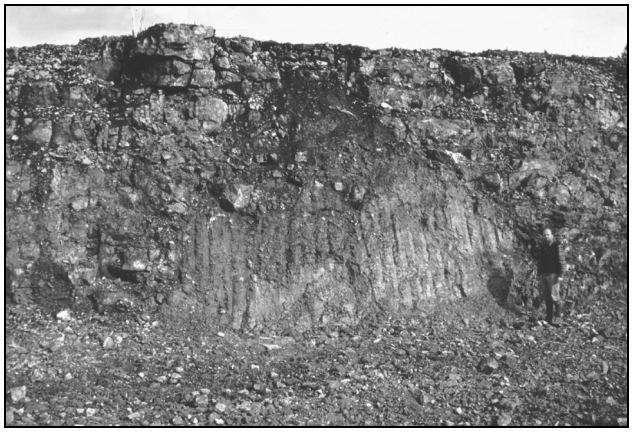
Posebna in pogosta kraška oblika so jame brez stropa. Ta, danes tudi pomembna površinska kraška oblika je že znan pojav, ki pa ni bil v celoti preučen. Posvečeno mu je bilo premalo pozornosti, saj je delež tovrstnih površinskih pojavov precej večji, kot je bilo domnevano. Število objav o jamah brez stropa je povezano z graditvijo novih odsekov avtocest (Knez & Šebela, 1994; Šebela & Mihevc, 1995; Slabe, 1996, 1997a, 1997b, 1998; Mihevc, 1996; Mihevc & Zupan Hajna, 1996; Kogovšek *et al.*, 1997; Mihevc *et al.*, 1998; Šebela *et al.*, 1999; Knez & Slabe, 2000, 2001, 2002, 2004a, 2004b, 2005). Oblika jame brez stropa je posledica vrste in oblike jame ter razvoja kraškega vodonosnika in njegovega površja v različnih geoloških, geomorfoloških, podnebnih in hidroloških razmerah. Izrazitost površinske oblike jame brez stropa pa je narekovana s hitrostjo odnašanja naplavin iz jame v primerjavi z nižanjem okolnega površja. Na površini razberemo prst in rastje ali pa pasove naplavine in sige, če je to počasno, ko pa je hitrejše, so jame brez stropa na kraškem površju podobne vrtačam, nizom vrtač ali pa so podolgovate zajede. Pogosto so splet različnih starih oblik, jam torej, in današnjega oblikovanje krasa z vrtačami in brezni.

Velik delež jam je bil zapolnjen z naplavinami. Največkrat so to poplavne drobnozrnate flišne naplavine, vmes pa so tudi plasti proda. Vzeli smo tudi vzorce naplavin za paleomagnetne raziskave in za naplavine v jamah pri Kozini in Divači smo ugotavljali, da so starejše olduvai dobe. Zato sklepamo, da so jame posledica messinske krize in so bile fosilizirane po ponovni zapolnitvi mediteranske kotanje z vodo, torej pred približno 5,2 milijona leti (Bosak *et al.*, 2000).

Skratka, jame brez stropa so vse bolj razločno berljiv pojav na kraškem površju, so pomemben del epikrasa in izjemna sled razvoja kraškega vodonosnika. Naši izsledki so koristni tudi pri načrtovanju različnih posegov v kras.

Po datacijah naplavin razbiramo najstarejša obdobja zakrasevanja Krasa in ugotavljamo, da so najstarejše jame na krasu precej starejše, kot so to predvidevali krasoslovci pred nami.

Sl. 7: Udor stropa jame med graditvijo useka pri počivališču pri Divači.
Fig. 7: A cave roof collapse during the construction of the roadcut near a rest place in the vicinity of Divača.



Sl. 8: V avtocestnem useku odkrita, s sedimenti v celoti zapolnjena jama.
Fig. 8: A cave discovered in the roadcut is completely filled up by sediments.

ZAKLJUČEK

Ugotavljamo, da je sodelovanje krasoslovcev pri graditvi avtocest na krasu koristno. Pomembno pa je, da se vključujemo tako v načrtovanje kot v graditev ter seveda tudi kasneje v spremljanje vplivov, ki jih imajo avtoceste na okolje, torej v celosten proces poseganja v občutljivo kraško pokrajino. Na ta način je ta lahko smiseln, ohranjamo naravno dediščino, poglabljamo temeljno znanje o nastanku in razvoju krasa in graditvi avtocest v tem svojevrstnem okolju. Poznamo več različnih vrst krasa in vsak zahteva svojstven pristop, zato mora biti sodelovanje med graditelji stalno in sprotno. V zadnjih desetih letih nam je ta spoznanja v Sloveniji v veliki meri uspelo uresničiti in sodelovanje med načrtovalci in graditelji cest ter krasoslovci nam je vzor tudi pri načrtovanju in izvedbi drugih posegov v kras.

KARSTOLOGICAL RESEARCH DURING THE CONSTRUCTION OF MOTORWAYS CROSSING THE SLOVENE KARST

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SUMMARY

Karstologists are taking part in motorway construction planned in the karst. In preliminary studies we select the most suitable alignments together with the planners and study, by regular karstological control during the construction, the newly discovered karst phenomena as well as help builders to overcome the karst properties; at the same time we try to preserve as many of these phenomena as possible. During the construction of the recent part of motorway, more than 350 caves opened on 60 km of the foreseen roadway. Different types of caves reflect the evolution of through-flow and outflow aquifer due to underground water table and karst surface lowering.

When planning and choosing the alternatives of laying out, one must consider geological, geomorhological, speleological and hydrogeological circumstances and the integrity of karst regions where motorways are constructed. We tried to avoid important karst phenomena, such as collapse dolines, large dolines, caves and karst walls, and by impermeable construction of a roadway tried to prevent the pollution flowing from it into underground waters. During the motorway construction, earthworks uncovered the karst surface and in places where the roadway was cut deeper into it or rocks excavated by tunnels, numerous karst caverns opened reflecting the development of aquifer. We investigated all the caves; we studied sediments and flowstone in them and attempted to preserve more important ones, considering that caves constitute a significant part of our natural heritage. This research supplemented new knowledge related to formation and evolution of karst. In particular important are new achievements related to roofless caves and to their shape on the karst surface, which can be usefully applied by planning new road sections. In view of the newly acquired experience, we may recognise them on the karst surface even before the earthworks started.

Key words: motorway construction, karst, karst cave, Classical Karst (Kras), Slovenia

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THE IMPORTANCE OF THE TIVAT SALINA (MONTENEGRO) FOR MIGRATING AND WINTERING WATERBIRDS, INCLUDING SOME NOTES ON PASSERINES

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ABSTRACT

During the 16 counts carried out between June 2003 and March 2006 along the main dam, 47 species of waterbirds and waders or a total of 111 bird species were recorded in the Tivat salina (Montenegro). Almost all waterbirds were found in the seaward section of the salina and along the coastline (33 ha). In view of the frequencies of their occurrence and maximum numbers/count, the Yellow-legged Gull (Larus michahellis), *Black-headed Gull* (L. ridibundus*), Eurasian Wigeon* (Anas penelope*) and Common Coot* (Fulica atra) *were the most abundant migrant and winter visitors. Among all waterbirds, evidence of nesting was found for the Little Ringed* (Charadrius dubius) *and Kentish Plovers* (Ch. alexandrinus) *only. During the spell of very low temperatures in January 2006, maximum numbers of > 1200 birds, mostly Yellow-legged Gulls and Common Snipes* (Gallinago gallinago), *or twice as many waterbirds in comparison to the former January counts, were present in the area. Low numbers of waterbirds in the salina are discussed in regard to the management plan, which is currently under consideration by the local authorities.*

Key words: waterbirds, waders, passerines, conservation, management plan, Tivat salina, Montenegro

IMPORTANZA DELLE SALINE DI TIVAT (MONTENEGRO) PER UCCELLI ACQUATICI MIGRANTI E SVERNANTI, INCLUSE NOTE SU PASSERACEI

SINTESI

Durante 16 censimenti effettuati fra giugno 2003 e marzo 2006 lungo l'argine principale delle saline di Tivat (Montenegro), sono state contate 47 specie di uccelli acquatici e trampolieri, ovvero 111 specie di uccelli in tutto. Quasi tutti gli uccelli acquatici sono stati avvistati nella sezione volta al mare delle saline e lungo la linea di costa (33 ha). In base alla frequenza d'avvistamento ed al numero di individui contati, le specie migranti e i visitatori invernali più abbondanti sono risultati: il gabbiano reale (Larus michahellis), *il gabbiano comune* (L. ridibundus), *il fischione (*Anas penelope*) e la folaga* (Fulica atra). *Fra tutte le specie di uccelli acquatici, con certezza nell'area nidificano il corriere piccolo* (Charadrius dubius) *ed il fratino* (Ch. alexandrinus). *Durante il periodo di temperature molto basse nel gennaio 2006, è stato contato il numero più alto di uccelli, più di 1200 esemplari, in prevalenza di gabbiano reale e beccaccino* (Gallinago gallinago), *ossia il doppio rispetto al mese di gennaio degli anni precedenti. Gli autori discutono del basso numero di uccelli acquatici nelle saline e del piano di gestione, attualmente in fase di preparazione presso le autorità locali.*

Parole chiave: uccelli acquatici, trampolieri, passeracei, tutela, piano di gestione, saline di Tivat, Montenegro

INTRODUCTION

Along the rocky shores of Dalmatian karst and its islands, most wetlands are restricted to a handful of river mouths and their alluvial lowlands. Some large wetland areas, which are of importance for wintering or migrating waterbirds using the Adriatic flyway, such as Vransko Jezero Nature Park and the Neretva river delta, are designated as Ramsar sites (Heath & Evans, 2000; Radović *et al.*, 2004). Even so, almost all estuaries, coastal lagoons, and inland marshes along the eastern coast of the Adriatic Sea have been heavily degraded by drainage, agricultural expansion and urbanization. In addition, the suitability of many wetlands for nesting or resting waterbirds and other wildlife is heavily impaired by unregulated or uncontrolled hunting and fishing (Hagemeijer *et al.*, 1994; Štumberger *et al.*, 2005).

Considering that many small wetland areas of the eastern Adriatic region are not covered by International Waterfowl Counts (IWC), information concerning their significance for waterbirds is mostly arbitrary or simply missing. Together with the delta of the Bojana/Buna River along the Albanian border and the marshlands of Buljarica Bay, the Tivat salina constitutes, in terms of its surface area, the most important wetlands along the coastline of Montenegro. Saveljić & Rubinič (2004) have published a list of 32 species of waterbirds and some raptors, which they recorded in the salina between 1999 and 2005. To evaluate the current importance of the area for migrating waterfowl, in addition to their list, waterbird counts are analysed herewith that have been performed by us in the Tivat salina since 2003. We hope that our data will be of certain help in the preparation of the management plan and in the evaluation regarding the implementation of various protection measures, as well as stimulate further monitoring in the area.

MATERIAL AND METHODS

Description of the study area

The salt-pans of Tivat are situated south of the city of Tivat (42°26′ N, 18°42′ E), approximately 16 km inland from the entrance to the Bay of Kotor (Boka Kotorska) on the east Adriatic coast. Above alluvial soils with > 3% salt in surface layers, the salt-pans of approximately 150 ha extend in the lowest part of Tivat Polje on the south-eastern fringes of the Bay of Tivat (Tivatski Zaljev), which forms the southernmost inlet of the Bay of Kotor (Magaš, 2002). The Tivat salt-pans (Solila) have been used for salt production since the $14^{th}/15^{th}$ century. During the early 1960s, the former salt-pans were renewed and adapted for industrial salt production (Cubrović, 2005). Although the modern salt-works have never been put into operation, the original marshlands were divided into artificial basins and cut from the south-west to the north-east by a 714 metres long dam in a smaller seaside and a much larger inland part. The seaward section along the shore-line is dominated by shallow waters and temporarily flooded mudflats. These are covered by almost closed stands of halophytic vegetation of Glasswort *(Salicornia europaea),* Perennial Glasswort *(Arthrocnemum* sp.) and Sea Rush *(Juncus maritimus).* As the inland section still receives some water by tidal waters through the main dam, the basins along the landward side of the main dam were during most of our visits partly covered by shallow waters, but dried out irregularly during late summer and autumn. They are covered by low sedges *(Carex),* softrush *(Juncus)* and scattered stands of other brackish and freshwater vegetation. Along the canals, narrow but dense stands of Common Reed *(Phragmites communis)* are thriving, while most of the smaller levees are heavily overgrown by high grasses, scrub and low bushes.

Methods

Between June 2003 and March 2006, we managed to visit the Tivat salina on 16 occasions. Depending on time schedules we performed, on our way to and from the Bojana/Buna delta, waterbird counts for EURONATUR (Radolfzell) at all times of the day (Tab. 1). Irrespective of day time, we standardized counts by counting all birds we could find in the seaward section of the salina by walking along a constant route of 714 m along the main dam from the road to Radovići to the dam's north-eastern end. Besides two Black-throated Divers *(Gavia arctica)* and a few scattered Great Cormorants *(Phalacrocorax carbo),* flocks of Yellow-legged Gulls *(Larus michahellis)* were noted in the coastal waters up to 1.5–2 km out to the Bay of Tivat during most counts. Due to human disturbances, many gulls constantly moved between the seaward section of the salina and the nearby refuse dumps of the city of Tivat. To overcome the problem of heavily fluctuating numbers of gulls during individual counts, we added all Yellow-legged Gulls, which were present in the bay or were seen moving between the salina, the bay or the nearby refuse dumps, to a single number/count (*cf.* footnotes in Table 2). Like in the seaward section, all birds that we could hear or find with the help of binoculars (10 x 40) and telescopes (30x, 20–60x) from the dam in the landward part of the salina were noted. Owing to the open terrain, our counts thus covered most birds present in the seaward section, whereas we most probably underestimated the numbers of smaller waterbirds, such as rails or some ducks, and of many passerines in the landward part of the salina. With a mean duration of 45.0 ± 17.2 min/count (95% confidence limits; n = 14), we spent > 570 min in the area (Tab. 1). For the comparison of the occurrence and numbers of different waterbird species, we calculated their frequencies of occur-

Tab. 1: Date, time and weather conditions during the 16 waterbird counts in the Tivat salina, June 2003 – March 2006.
Tab. 1: Datum, čas in vremenske razmere med 16 štetji vodnih ptic v Tivatskih solinah med junijem 2003 in marcem 2006.

No.	Date	Time of day (CET)	Weather conditions	Observer
1	10.6.2003	16:00–16:30	clouds 0/8, wind 0	P. Sackl, B. Štumberger
2	31.10.2003	13:30–14:10	clouds 1/8, wind 2	P. Sackl, B. Štumberger, J. Smole
3	15.11.2003	10:30–10:50	clouds 8/8, wind 1	P. Sackl, J. Smole
4	31.1.2004	–	–	P. Sackl, B. Štumberger
5	10.4.2004	12:55–13:15	clouds 8/8, windless, slight rain	P. Sackl, B. Štumberger
6	17.4.2004	–	–	P. Sackl, B. Štumberger
7	12.10.2004	17:40–18:20	clouds 8/8, wind 1–2, after rain	T. Lončar, P. Sackl, J. Smole
8	26.10.2004	11:05–11:30	clouds 0/8 – 2/8, wind 0–1	T. Lončar, P. Sackl, J. Smole
9	15.1.2005	15:50–16:20	clouds 2/8, wind 2–3, 3 °C	P. Sackl, B. Štumberger
10	22.1.2005	17:45–18:00	clouds 6/8, wind 1–2	P. Sackl, B. Štumberger
11	23.4.2005	9:15–10:03	clouds 1/8, wind 1, 10 °C	I. Geister, B. Štumberger
12	5.5.2005	11:10–11:45	clouds 8/8, wind 0–1, slight rain	T. Lončar, P. Sackl
13	29.10.2005	14:40–16:40	clouds 0/8, wind 0, very warm	T. Lončar, P. Sackl
14	5.11.2005	12:35–13:30	clouds 1/8, wind 0–1, warm	T. Lončar, P. Sackl
15	21.1.2006	15:00–15:57	clouds 8/8, wind 0	P. Sackl, B. Štumberger
16	21.3.2006	11:20–12:55	clouds 8/8, wind 0, ca. 15 °C	D. Denac, B. Štumberger

rence (= % of counts when a species was present) and their respective maximum numbers during all counts (= visits). Statistical analyses were performed with non-parametric tests by using the statistical package SPSS, version 13.0 (2004).

RESULTS

Species diversity

Since June 2003, we noted 104 bird species in the salina. Their status and numbers per count are given in Table 2. Our list includes 46 Passeriformes (44.2%), while according to the subject and field methods of our study the larger portion of 58 species or 55.8% of all species recorded belong to the Non-Passeriformes. Including 7 species of waterbirds cited by Saveljić & Rubinič (2004), which we missed in Tivat, 65 Non-Passeriformes (= 58.6%) or a total of 111 species are currently documented for the area. Most of the species found in the salina are classified as passage migrants (58/55.8%) or winter visitors (13/12.5%). The rest is made up of confirmed or possible breeders (17/16.3%) and species (16/15.4%) that presumably nest in adjoining habitats and visit the salt-pans for feeding or outside the breeding season (Tab. 2). While 27 species or 58.7% of all passerines are confirmed or probable breeders, evidence of nesting, as far as Non-Passeriformes are concerned, was found for the Little Ringed *(Charadrius dubius)* and Kentish Plovers *(Ch. alexandrinus)* only.

Waterbirds

In addition to waders, we noted 30 waterbird species (including Common Kingfisher *Alcedo atthis*), which are usually covered by the IWC scheme (Tab. 2). In all waterbird species, the frequencies of their occurrence and respective maximum numbers are positively correlated (r_P = 0.36, P = 0.054, n = 30); *i.e.* the species, which we saw more regularly in the salina, tend to appear in larger numbers. Besides the small numbers of divers (Gaviidae), grebes (Podicipedidae), cormorants (Phalacrocoracidae) and some rare passage migrants, the resident Yellow-legged and wintering Black-headed Gulls *(Larus ridibundus)* were, together with Eurasian Wigeon *(Anas penelope)*, Common Coot *(Fulica atra)*, Grey Heron *(Ardea cinerea)* and Little Egret *(Egretta garzetta)*, the most abundant migrant and winter visitors. Coinciding with spring migration of the species, large numbers of Garganey *(Anas querquedula)* were present in March 2006 (Tab. 2). Altogether, these species amounted to 94.4% of all waterbirds counted (n = 4767). Little Grebe *(Tachybaptus ruficollis)*, Great White Egret *(Egretta alba)* and Common Kingfisher, which overwinter in low numbers of 1–6 individuals (ind.), occurred in high frequency (Tab. 2).

Numbers of species in all winter periods since 2003 show no clear seasonal trend (Fig. 1), whereas the population numbers increased from autumn till January (Fig. 2), when large numbers of Eurasian Wigeon, Common Coots and Black-headed Gulls were concentrated in the shallowly flooded areas along the shore-line.

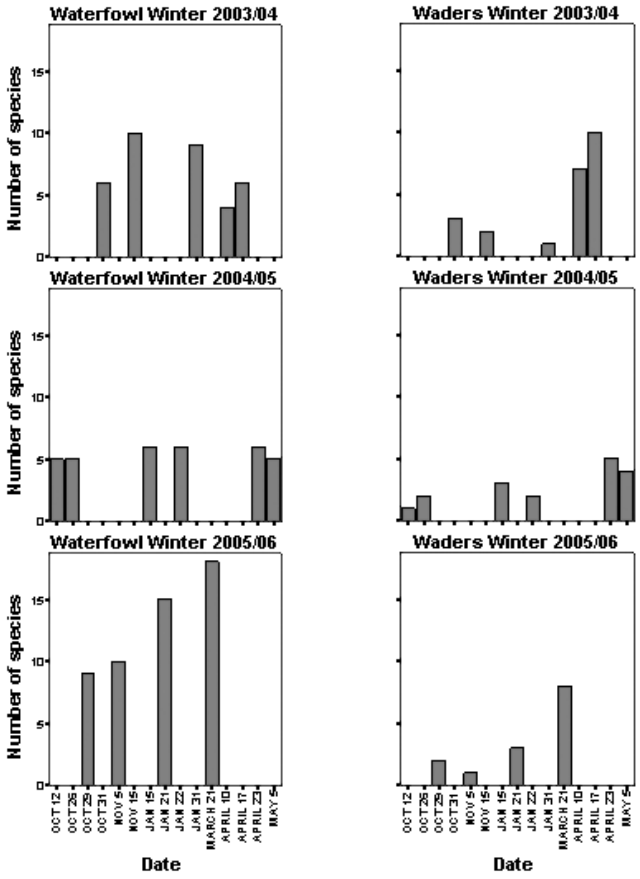

Fig. 1: Numbers of waterbird and wader species recorded during waterbird counts in the Tivat salina, June 2003–March 2006.
Sl. 1: Število vodnih ptic in pobrežnikov, zabeleženih med štetjem ptic Tivatskih solin v obdobju med junijem 2003 in marcem 2006.

In contrast to the moderately warm winters 2003/04 - 2004/05, heavy winds ('bora') and temperatures below freezing prevailed during our visit in January 2006 along the eastern Adriatic's coast. While the Tivat salina was free of ice during our visit on January 21st, two days later the shallow inlet and the greater part of the Viluni Lagoon on the Albanian coast as well as most basins in the salt-works of Ulcinj (Montenegro) were covered with ice, with dozens of starving and freshly frozen Common Coots scattered across the ice sheet of the Viluni Lagoon. In Tivat, the numbers of species as well as population numbers of January counts were not significantly different between the three winter periods since 2003 (Kruskal-Wallis tests, numbers of species: $\chi^2 = 3.00$, P = 0.22; numbers of birds: $\chi^2 = 1.80$, P = 0.41; in both cases df = 2), although peak numbers of > 1200 waterbirds, *i.e.* in comparison to former January counts, twice as many birds, and additional numbers of 6–9 waterbird species, were present in January 2006 (Figs. 1, 2). 83.5% of all waterbirds, which were present on January 21st 2006, were Yellow-legged Gulls that were resting along shore-line or foraging at the nearby refuse dumps of the city of Tivat (Tab. 2).

Waders

Of 507 waders of the suborder Charadrii, which we have counted since 2003, Common Snipe *Gallinago gallinago* (n = 187) and Common Redshank *Tringa totanus* (n = 174) were most abundant in terms of their numbers. Regarding the frequencies of occurrence, on the other hand, Common Redshank (81.3%), Eurasian Curlew *Numenius arquata* (37.5%), Common Snipe (31.3%), Wood Sandpiper *Tringa glareola* (31.3%) and Greenshank *T. nebularia* (25.0% of all counts) were the most common species. All other waders occurred in frequencies < 20% (Tab. 2). Like in waterbirds, maximum numbers/count and frequencies of occurrence are positively correlated ($r_P = 0.48$, P = 0.054, n = 17). In contrast to Common Snipes, which were seen in large numbers only in January and March 2006, wintering Common Redshanks (2–27 ind./count) and Eurasian Curlews

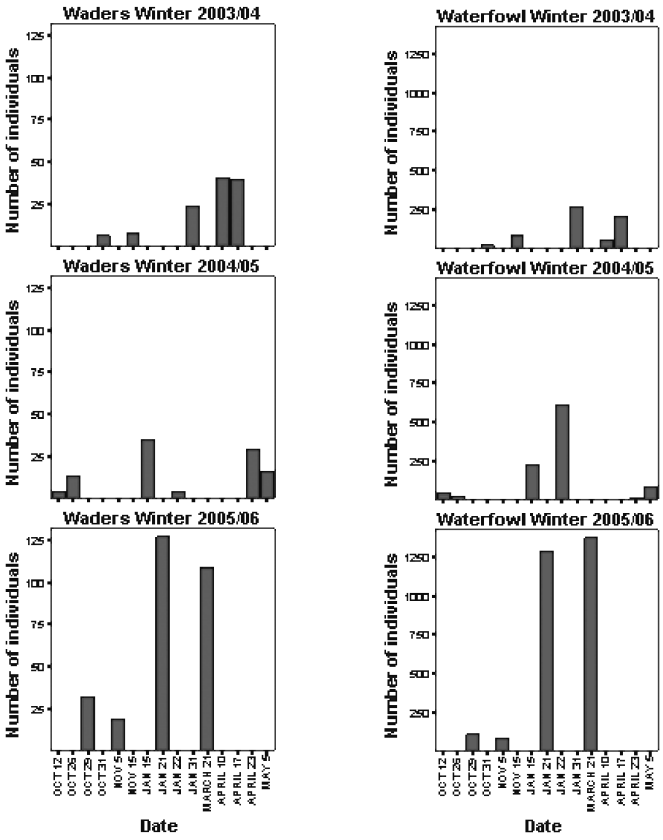

Fig. 2: Population numbers of waterbirds and waders recorded during waterbird counts in the Tivat salina, June 2003-March 2006. (Note different scales of ordinates for waterbirds and waders.)
Sl. 2: Skupno število vodnih ptic in pobrežnikov, zabeleženih med štetjem ptic v Tivatskih solinah v obdobju med junijem 2003 in marcem 2006. (Pazi na različno velike skale na y-oseh.)

(1–3 ind./count) were present in low numbers, although during most visits between October and January (Tab. 2). In waders, numbers of species peaked during spring migration (March-May), whereas numbers of birds showed no clear seasonal trend (Figs. 1, 2). Like in waterbirds, our data show no statistically significant effect of the unusually low temperatures in January 2006 (Kruskal-Wallis for January counts/winter; number of species: $\chi^2 = 2.25$, P = 0.33; numbers of birds: $\chi^2 = 1.80$, P = 0.41; in both cases df = 2). With a total of three species, we noted similar numbers of species like during the former January counts (1–3 species), while the peak numbers of waders in January and March 2006 were made up by Common Snipes, which amounted to 85.8% of all waders we counted on January 21st, 2006 (Tab. 2).

Passerines

In view of the species and numbers, we noted during the waterbird counts that the Tivat salina is an important site for some migrating and wintering passerines. Apart from flocks of migrating Sand Martins *(Riparia riparia)* and Barn Swallows *(Hirundo rustica)*, we counted a minimum of 141 Whinchats *(Saxicola rubetra)* on April 17th, 2004. With a strip of 100-200 metres, which we presumably covered by counting along both sides of the main dam, we roughly estimated the densities of 96.8-193.7 Whinchats/10 ha for that day. While we saw small numbers of Water *(Anthus spinoletta)* and Meadow Pipits *(A. pratensis)* between October and January, at least 53 Meadow Pipits were feeding on mudflats in the seaward section of the salina in January 2006. Passage and winter visitors, which were noted regularly during winter, were Hedge Accentor *(Prunella modularis)*, Robin *(Erithacus rubecula)*, and Great Grey Shrike *(Lanius excubitor)* (Tab. 2). In late April 2005, we noted a singing Reed Warbler *(Acrocephalus scirpaceus)*, whereas in May of the same year we saw a displaying male Zitting Cisticola *(Cisticola juncidis)* in the landward basins beside the main dam. Minimum numbers of other species of conservation concern, which we noted, were 3 breeding pairs (bp.) of Tawny Pipit *(Anthus campestris)*, 2-3 singing Great Reed

Warblers *(Acrocephalus arundinaceus)*, 1-3 singing Corn Buntings *(Miliaria calandra)*, 1–3 bp. of Red-backed Shrike *(Lanius collurio)* and at least 1 bp. of Whoodchat Shrike *(L. senator)*.

DISCUSSION

In comparison to other coastal wetlands of the central and eastern Mediterranean, the numbers of waterbirds and waders appear to be low in the Tivat salina, although throughout the Mediterranean region salinas are important for a variety of breeding, over-wintering and migrating waterbirds (Petanidou, 1977; Anonymus, 1996). With only two visits during the breeding season in June 2003 and early May 2005, our data are largely restricted to the winter months between October and April. In the same way, Saveljić & Rubinič (2004) visited the salina only during the migration and winter periods. Thus, current information concerning the potential of the area for breeding waterfowl, waders and other birds is arbitrary. Concerning the numbers of resting and wintering waterbirds, we noted in Tivat, besides migrating Garganeys, wintering Common Snipes, Black-tailed Godwits *(Limosa limosa)* and resident Yellow-legged Gulls, the species whose maximum numbers exceed 100 birds, *i.e.* Eurasian Wigeon, Common Coot and Black-headed Gull, belong to the most abundant species of waterbirds, which overwinter in maximum totals of over one million birds throughout the Western Palaearctic (Gilissen *et al.*, 2002; Saveljić & Rubinič, 2004). According to the extent of the area, population numbers of most waterbirds and waders are low in Tivat. Currently, no species reaches the 1% level of Ramsar criterion 3c (Wetlands International, 2002).

The low numbers of waterbirds in the Tivat salina may result from different reasons. In general, most of the eastern Adriatic coastline consists of rocky shores. Together with low tidal amplitudes < 30 cm, this appears to prevent many waterbird species, in particular waders and some ducks preferring shallow waters, to winter in large numbers along the Dalmatian coast (*e.g.* Smit, 1986; Stipčević, 1997). On the other hand, the area of permanently shallow waters and mudflats in Tivat, which is most suitable for feeding or resting waterbirds, is currently restricted to a narrow strip along the shoreline. With the erection of the main dam, the much bigger landward part of the salt-pans was cut off from the sea. The area of permanent water and low stands of halophytic vegetation on the seaward side of the main dam currently covers 33 ha, *i.e.* less than a third of the entire area of the salt-pans. With the exception of a few Little Egrets, which fed in the partly flooded basins of the landward section in spring 2004, we saw all waterbirds in the permanently flooded seaward part of the salina and along the coastline.

In addition, as a result of excessive shooting and hunting throughout the Balkans, all waterfowl, herons and waders are extremely frightened, as indicated by flight distances from people and boats of ≥ 300 metres (Sackl & Štumberger, *unpubl.*). During our visits, we regularly found used cartridges along the main dam in Tivat. In addition, the main dam is regularly used by the locals, while fishermen and oyster-farmers operate year-round < 100-200 m from the shore-line, forcing herons, ducks and other waterbirds along coastline regularly to take wing, although they are not shooting or carrying shot-guns. Therefore, we recommend re-evaluating whether the 500–520 m wide strip between the shoreline and the main dam, which according to the actual management plan is proposed to be prohibited for people, is wide enough for undisturbed feeding and resting of waterbirds.

Finally, to combat infestations of mosquitoes and other pests, spraying of pesticides is practiced during the spring and summer (Fig. 3). This may reduce the availability of insects and other invertebrate prey for birds. Although few studies have been made in the Mediterranean region, methoprene, an insect growth regulator used to combat mosquitoes, and other chemicals used for pest control, are known to reduce populations of dragonflies (Odonata), chironomid Diptera and other insect larvae (review in Grillas *et al.*, 2004). In addition to the high level of human disturbances, this may explain why we have found such low numbers of nesting waders and passerines in the salina (Tab. 2).

According to its extents and locality in the low-lying depression along the southern shore-line of Boka Kotorska, which is surrounded by high mountains and largely undisturbed scrub-lands, the Tivat salina may be suitable for much larger numbers of waterbirds (Saveljić & Rubinič, 2004). The potential of the area is illustrated by the

Fig. 3: Infestations of mosquitoes are controlled with insecticides along the main dam of the Tivat salina, 23rd April, 2005. (Photo: B. Štumberger)
Sl. 3: Vzdolž glavnega nasipa v Tivatskih solinah se zatiranja komarje zalege lotevajo z insekticidi, 23. april 2005. (Foto: B. Štumberger)

relatively large number of 47 species of waterbirds and waders, which we have noted since 2003. In addition, the salina appears to function as a refuge for waterbirds wintering along the Dalmatian coast and its Balkan hinterlands during the spells of low temperatures and heavy winds. Prolonged periods of severe weather, which force wintering waterfowl to search for open waters, are well known to trigger irregular and sometimes massive movements of waterbirds, like geese, Common Teal *(Anas crecca)* and other ducks, in northern Europe (Kear, 2005). Although our data are not statistically significant, this is illustrated by the large numbers of Yellow-legged Gulls, Common Snipes, Spotted Redshank *(Tringa erythropus)* and other waterbirds, which were present at Tivat in late January 2006. Besides many Yellow-legged Gulls, which over-winter mainly along the coastline, heavy winds and persistent frost may have forced some ducks and waders, which normally winter in inland wetlands of the western Balkans, to search for ice-free conditions along the coast of the eastern Adriatic.

The potential of the salina and its wider environment for birds and other wildlife is further documented by nine species of raptors and a variety of passerines, which we have noted during the migration and winter periods (Tab. 2). In addition, the salina of Tivat, where Stojnić (2004) found the first evidence of nesting in August 2004, is the only known nesting site of Zitting *Cisticola* in Montenegro apart from the Bojana/Buna delta. In the same way, Reed Warblers nest rarely and very dispersedly along the Dalmatian coast (Schulze-Hagen, 1997; Rucner, 1998); although the singing male we noted in late April 2005 could have been a migrant visitor as well. Population numbers of some passerines, such as Tawny Pipit, Great Reed Warbler, Red-backed Shrike and Corn Bunting, which apparently nest more regularly in the salina, show long-term decline in most central and western European countries (Burfield & van Bommel, 2004; Bauer *et al.*, 2005). In addition, outside our waterbird counts we saw an adult Gull-billed Tern *(Gelochelidon nilotica)* on the adjoining airfield of Tivat airport on July 12th, 2002, and since 2003 we have found three Golden Jackals *(Canis aureus)* killed by traffic along the main road E-65 between Tivat airport and the crossroads to the village of Dub.

The Regional Tourist Master Plan proposes a buffer zone around the salina, which will function as a landscape park and where main recreational activities will take place (DEG, 2003), while the seaward section of the salina has been protected by the national law since autumn 2005 (Saveljić & Rubinič, 2004). This and a management plan currently under consideration by the local authorities is a good chance for restoring the Tivat salina as a stop-over and nesting site for many waterbirds along the Adriatic flyway.

Tab. 2: Bird species, their status and respective numbers noted during the 16 waterbird counts in the Tivat salina (Montenegro), June 2003 – March 2006. Maximum numbers per species during all counts are indicated by bold numbers.
Legend: RB = resident breeder; BM = breeding migrant; BM? = possible or probably breeding migrant; (RB) and (BM) = resident breeder or breeding migrant possible or probably breeding in adjoining habitats; PM = (regular) passage migrant; WV = winter visitor; WV? = probably wintering; bp = breeding pair; m = male; s = singing male; Occ. (%) = numbers/percentage of counts when a species was present (frequency of occurrence).
Tab. 2: Vrste ptic, njihov status in število posameznih vrst, ugotovljenih med 16 štetji vodnih ptic v Tivatskih solinah (Črna gora) med junijem 2003 in marcem 2006. Maksimalno število vrste med vsemi štetji je označeno s krepko natisnjenimi številkami.
Legenda: RB = gnezdeča stalnica; BM = seleča se gnezdilka; BM? = možna ali verjetna seleča se gnezdilka; (RB) in (BM) = gnezdeča stalnica ali seleča se gnezdilka, ki morda ali verjetno gnezdi v sosednjih habitatih; PM = (redni) preletnik; WV = zimski gost; WV? = verjetni prezimovalec; bp = gnezdeči par; m = samec; s = pojoči samec; Occ. (%) = število/odstotek štetij, ko je bila vrsta opažena v solinah (frekvenca pojavljanja).

Species	Status	10.06. 03	31.10. 03	15.11. 03	31.01. 04	10.04. 04	17.04. 04	12.10. 04	26.10. 04	15.01. 05	22.01. 05	23.04. 05	05.05. 05	29.10. 05	05.11. 05	21.01. 06	21.03. 06	Occ. (%)
Black-throated Diver *Gavia arctica*	WV															**2**		1 / 6.3%
Little Grebe *Tachybaptus ruficollis*	PM/WV			2	2							1		3	3	**6**	**6**	7 / 43.8%
Great Crested Grebe *Podiceps cristatus*	WV				**6**					1						3	4	4 / 25.0%
Black-necked Grebe *Podiceps nigricollis*	WV															**7**		1 / 6.3%
Great Cormorant *Phalacrocorax carbo*	WV				3						4					2	**54**	4 / 25.0%
Pygmy Cormorant *Phalacrocorax pygmeus*	PM		7	1										3	**20**			4 / 25.0%
Squacco Heron *Ardeola ralloides*	PM												**1**					1 / 6.3%

Species	Status	10.06. 03	31.10. 03	15.11. 03	31.01. 04	10.04. 04	17.04. 04	12.10. 04	26.10. 04	15.01. 05	22.01. 05	23.04. 05	05.05. 05	29.10. 05	05.11. 05	21.01. 06	21.03. 06	Occ. (%)
Little Egret *Egretta garzetta*	PM		2	1		**99**	28	3	1			14	3	2	1		3	11 / 68.8%
Great White Egret *Egretta alba*	PM/WV	1						1	1	1				**2**	**2**	1	1	8 / 50.0%
Grey Heron *Ardea cinerea*	PM/WV	4	2	8	5	**83**	19	1	3	12	1	4		8	6	5	47	15 / 93.8%
Glossy Ibis *Plegadis falcinellus*	PM					**1**												1 / 6.3%
Eurasian Spoonbill *Platalea leucorodia*	PM					2	1										**7**	3 / 18.8%
Mute Swan *Cygnus olor*	PM																**2**	1 / 6.3%
Common Shelduck *Tadorna tadorna*	PM			**1**														1 / 6.3%
Eurasian Wigeon *Anas penelope*	PM/WV			27	17					102	**276**			21	33	69	18	8 / 50.0%
Common Teal *Anas crecca*	WV															**16**	6	2 / 12.5%
Mallard *Anas platyrhynchos*	WV									2						**17**		2 / 12.5%
Garganey *Anas querquedula*	PM											2					**588**	2 / 12.5%
Northern Shoveler *Anas clypeata*	PM												1				**20**	2 / 12.5%
Northern Pintail *Anas acuta*	PM/WV?																**15**	1 / 6.3%
Common Pochard *Aythya ferina*	WV															**7**		1 / 6.3%
Ferruginous Duck *Aythya nyroca*	PM																**14**	1 / 6.3%
Osprey *Pandion haliaetus*	PM																**1 ad**	1 / 6.3%
Marsh Harrier *Circus aeruginosus*	PM					2											**5**	2 / 12.5%
Hen Harrier *Circus cyaneus*	WV															**1**		1 / 6.3%
Montagu's Harrier *Circus pygargus*	PM/WV					**3**				1								2 / 12.5%
Northern Goshawk *Accipiter gentilis*	PM		**1**															1 / 6.3%
Eurasian Sparrowhawk *Accipiter nisus*	PM/WV		1													**2**		2 / 12.5%
Common Buzzard *Buteo buteo*	PM/WV		1		**3**				1						2		1	5 / 31.3%
Red-footed Falcon *Falco vespertinus*	PM						**2**											1 / 6.3%
Common Kestrel *Falco tinnunculus*	(RB)																**1m**	1 / 6.3%
Water Rail *Rallus aquaticus*	PM/WV?		**1**												**1**			2 / 12.5%
Common Coot *Fulica atra*	WV			22	68					100	**196**					69	59	6 / 37.5%
Black-winged Stilt *Himantopus himantopus*	PM					**12**	1						3					3 / 18.8%
Little Ringed Plover *Charadrius dubius*	BM/PM	1 bp				4	**9**											3 / 18.8%
Kentish Plover *Charadrius alexandrinus*	BM?/PM					**1 bp**												1 / 6.3%
Grey Plover *Pluvialis squatarola*	PM												**2**					1 / 6.3%
Northern Lapwing *Vanellus vanellus*	PM		1											5			**6**	3 / 18.8%
Ruff *Philomachus ugnax*	PM						**6**										1	2 / 12.5%
Common Snipe *Gallinago gallinago*	PM/WV					1				19		8				**109**	50	5 / 31.3%
Whimbrel *Numenius phaeopus*	PM					**3**												1 / 6.3%
Eurasian Curlew *Numenius arquata*	PM/WV		1	1		**3**			**3**	**3**	2							6 / 37.5%
Dunlin *Calidris alpina*	PM																**1**	1 / 6.3%

Species	Status	10.06. 03	31.10. 03	15.11. 03	31.01. 04	10.04. 04	17.04. 04	12.10. 04	26.10. 04	15.01. 05	22.01. 05	23.04. 05	05.05. 05	29.10. 05	05.11. 05	21.01. 06	21.03. 06	Occ. (%)
Spotted Redshank *Tringa erythropus*	PM/WV															**11**		1 / 6.3%
Common Redshank *Tringa totanus*	PM/WV		4	6	23	5		4	10	13	2		10	27	19	7	**44**	13 / 81.3%
Marsh Sandpiper *Tringa stagnatilis*	PM					**5**	1										2	3 / 18.8%
Greenshank *Tringa nebularia*	PM					1	1					1	**13**					4 / 25.0%
Green Sandpiper *Tringa ochropus*	PM						1					**3**					2	3 / 18.8%
Wood Sandpiper *Tringa glareola*	PM					3	**21**					4	1				3	5 / 31.3%
Common Sandpiper *Actitis hypoleucos*	PM		**1**										**1**					2 / 12.5%
Black-headed Gull *Larus ridibundus*	PM/WV			2	**152**				11		128			7	4	8		7 / 43.8%
Yellow-legged Gull *Larus michahellis*	(RB)			19	3	12	1	30			2	54	ca. 63[1]	min. 60	11	**1075**[2]	525[5]	12 / 75.0%
Gull-billed Tern *Gelochelidon nilotica*	PM												**2**					1 / 6.3%
Sandwich Tern *Sterna sandvicensis*	PM/WV?																**1**	1 / 6.3%
Caspian Tern *Sterna caspia*	PM					**3**												1 / 6.3%
Alpine Swift *Tachymarptis melba*	(BM)	**ca. 100**																1 / 6.3%
Common Kingfisher *Alcedo atthis*	PM/WV		1	1	1			**4**	1					1	2	1	1	9 / 56.3%
Hoopoe *Upupa epops*	(BM)/PM											**1**						1 / 6.3%
Sky Lark *Alauda arvensis*	PM		**1**															1 / 6.3%
Sand Martin *Riparia riparia*	PM					> 20						**120**	ca. 20					3 / 18.8%
Barn Swallow *Hirundo rustica*	(BM)/PM					**> 250**							2	2			16	4 / 25.0%
Red-rumped Swallow *Hirundo daurica*	BM	**2 (= 1 bp)**											**2 (= 1 bp)**					2 / 12.5%
House Martin *Delichon urbicum*	(BM)												**6**					1 / 6.3%
Tawny Pipit *Anthus campestris*	BM	**3 bp**					**3s**											2 / 12.5%
Tree Pipit *Anthus trivialis*	PM		**2**															1 / 6.3%
Meadow Pipit *Anthus pratensis*	WV			1						15					min. 1	**53**	9	5 / 31.3%
Water Pipit *Anthus spinoletta*	PM/WV		2		2									**min. 2**				3 / 18.8%
Yellow Wagtail *Motacilla flava*	PM						13[3]					**25**						2 / 12.5%
Pied Wagtail *Motacilla alba*	(BM)/PM							**min. 3**										1 / 6.3%
Hedge Accentor *Prunella modularis*	WV			1	4			**5-10**								1	1	5 / 31.3%
Robin *Erithacus rubecula*	PM/WV		ca. 50	2	1			**> 50**						> 10	> 10	2	3	8 / 50.0%
Rufous Nightingale *Luscinia megarhynchos*	BM						1s					**3s**	1s					3 / 18.8%
Black Redstart *Phoenicurus ochruros*	PM/(RB)																**1**	1 / 6.3%
Whinchat *Saxicola rubetra*	PM		1				**141**						5				5	4 / 25.0%
Common Stonechat *Saxicola torquata*	PM		6												2		**27**	3 / 18.8%
Northern Wheatear *Oenanthe oenanthe*	BM?/PM					**> 10**						1					2m	3 / 18.8%
Black-eared Wheatear *Oenanthe hispanica*	BM?/PM					**1**												1 / 6.3%
Blackbird *Turdus merula*	RB						1		1			**2s**						3 / 18.8%

Species	Status	10.06. 03	31.10. 03	15.11. 03	31.01. 04	10.04. 04	17.04. 04	12.10. 04	26.10. 04	15.01. 05	22.01. 05	23.04. 05	05.05. 05	29.10. 05	05.11. 05	21.01. 06	21.03. 06	Occ. (%)
Song Thrush *Turdus philomelos*	WV				**3**												1	2 / 12.5%
Fieldfare *Turdus pilaris*	PM/WV?																**1**	1 / 6.3%
Cetti's Warbler *Cettia cetti*	RB					1s	1s					**2s**	1s	1s				5 / 31.3%
Zitting Cisticola *Cisticola juncidis*	RB?												**1s**					1 / 6.3%
Reed Warbler *Acrocephalus scirpaceus*	BM?											**1s**						1 / 6.3%
Great Reed Warbler *A. arundinaceus*	BM	2s											**3s**					2 / 12.5%
Subalpine Warbler *Sylvia cantillans*	BM					**2 (1s)**						**2s**	1s				1m	4 / 25.0%
Barred Warbler *Sylvia nisoria*	PM		**1**															1 / 6.3%
Common Whitethroat *Sylvia communis*	PM						**4**											1 / 6.3%
Common Chiffchaff *Phylloscopus collybita*	PM		**4**												1			2 / 12.5%
Firecrest *Regulus ignicapillus*	PM			**1**														1 / 6.3%
Blue Tit *Parus caeruleus*	(RB)/WV				**6**												1	2 / 12.5%
Red-backed Shrike *Lanius collurio*	BM	**3 bp**											1m					2 / 12.5%
Great Grey Shrike *Lanius excubitor*	WV				**1**			**1**	**1**		**1**			**1**				5 / 31.3%
Woodchat Shrike *Lanius senator*	BM?					**2**	1						**2**					3 / 18.8%
Eurasian Jay *Garrulus glandarius*	(RB)							**1**										1 / 6.3%
Magpie *Pica pica*	(RB)											**3**						1 / 6.3%
Carrion Crow *Corvus corone*	(RB)				**3**			1						2		2		4 / 25.0%
Common Starling *Sturnus vulgaris*	PM/WV													**11**				1 / 6.3%
House Sparrow *Passer domesticus*	(RB)				**2**													1 / 6.3%
Spanish Sparrow *Passer hispaniolensis*	RB	**1m**[4]																1 / 6.3%
Common Chaffinch *Fringilla coelebs*	(RB)																**3**	1 / 6.3%
Greenfinch *Carduelis chloris*	(RB)														**1m**			1 / 6.3%
Goldfinch *Carduelis carduelis*	(RB)			**1**											**1**			2 / 12.5%
Common Reed Bunting *Emberiza schoeniclus*	(RB)/WV ?																**2**	1 / 6.3%
Corn Bunting *Miliaria calandra*	RB	**3s**				1s	1s						2s					4 / 25.0%

[1, 2, 5] including ca. 60, ca. 700 and 450 ind. feeding at nearby refuse dump

[3] all males *M. f. cinereocapilla*

[4] displaying male

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POMEN TIVATSKIH SOLIN (ČRNA GORA) ZA PREZIMUJOČE IN SELEČE SE VODNE PTICE, IN NEKAJ OPAŽANJ O TAMKAJŠNJIH PEVKAH

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POVZETEK

Tivatske soline so glede na površino (150 ha), ki jo pokrivajo, med najpomembnejšimi mokrišči na črnogorski obali. Z namenom, da se dokumentira njihov današnji pomen za prezimujoče in seleče se vodne ptice, je bilo med junijem 2003 in marcem 2006 opravljenih 16 štetij vzdolž solinskega glavnega nasipa (714 m). Štetja so pokrivala celotni priobalni del solin in obrežje samo (33 ha), kjer se je zadrževalo največ vodnih ptic. V neenakomerno posušenih solnih bazenih na drugi strani glavnega nasipa je bilo zabeleženih zelo malo vrst vodnih ptic.

Skupaj je bilo zabeleženih 104 vrst ptic. Vključno s 7 vrstami, ki jih za to območje v svojem seznamu, ki vsebuje tudi vodomca (Alcedo atthis)*, navajata Saveljić & Rubinič (2004), je bilo v obravnavanem območju doslej ugotovljenih 47 vrst vodnih ptic oziroma 111 vrst vseh ptic. Najštevilnejši med njimi, s 94,4% vseh preštetih vodnih ptic (n = 4767), so bili tod prebivajoči rumenonogi galebi* (Larus michahellis) *in prezimujoči rečni galebi* (L. ridibundus)*, njim pa so sledile reglje* (Anas querquedula)*, žvižgavke* (A. penelope)*, črne liske* (Fulica atra)*, sive čaplje* (Ardea cinerea) *in male bele čaplje* (Egretta garzetta)*. Poleg teh vrst tu redno, a v manjšem številu od 1 do 6 osebkov, prezimujejo mali ponirki* (Tachybaptus ruficollis)*, velike bele čaplje* (Egretta alba) *in vodomci. Najštevilnejši med skupaj 17 vrstami pobrežnikov so bili glede na frekvenco pojavljanja in število osebkov, preštetih med vsemi popisi, rdečenogi martinci* (Tringa totanus)*, veliki škurhi* (Numenius arquata)*, kozice* (Gallinago gallinago)*, močvirski martinci* (T. glareola) in zelenonogi martinci (T. nebularia). *Kar zadeva vodne ptice, sta v preučevanem območju zanesljivo gnezdila le mali* (Charadrius dubius) *in beločeli deževnik* (Ch. alexandrinus)*. V obdobju neobičajno hladnega vremena in močnih vetrov proti koncu januarja 2006 je bilo v obravnavanem območju zabeleženo maksimalno število (> 1200) ptic, predvsem rumenonogih galebov (83,5%), ali kar dvakrat več kot med januarskimi štetji v prejšnjih letih. Čeprav se število vseh ptic statistično ne razlikuje od prejšnjih januarskih štetij, pa veliko število vodnih ptic, preštetih januarja 2006, govori o dejstvu, da imajo soline funkcijo zatočišča za vodne ptice, ki v obdobjih hudih vremenskih razmer prezimujejo vzdolž vzhodne jadranske obale (rumenonogi galeb) in v njenem balkanskem zaledju (kreheljc, mlakarica, kozica). O ornitološkem potencialu območja nadalje govorijo visoke številke tam počivajočih repaljščic* (Saxicola rubetra) *v aprilu 2004 kot tudi dokazi o gnezdenju rjave cipe* (Anthus campestris)*, srpične trstnice* (Acrocephalus scirpaceus)*, rakarja* (A. arundinaceus)*, brškinke* (Cisticola juncidis)*, rjavega* (Lanius collurio) *in rjavoglavega srakoperja* (L. senator).

Razmeroma nizko število vodnih ptic v Tivatskih solinah je lahko posledica nenadzorovanega lova, postavitve nasipa v šestdesetih letih prejšnjega stoletja, ki je otežil povezavo med kopenskim delom solin in morjem, in uporabe pesticidov za uničevanje komarjev v spomladanskih in poletnih mesecih. Upravljalski program, ki ga prav zdaj pripravljajo lokalne oblasti, je dobra priložnost, da se soline vnovič vzpostavijo kot postajališče in gnezdišče za vodne ptice. Toda glede na pridobljene rezultate bi bilo treba na novo oceniti, ali je predlagano osrednje območje vzdolž obale, ki je zdaj zaščiteno pred vznemirjanjem, ki ga povzroča človek, dovolj široko za nemoteno prehranjevanje in počitek vodnih ptic.

Ključne besede: vodne ptice, pobrežniki, pevke, varstvo, načrt upravljanja, Tivatske soline, Črna gora

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DELO NAŠIH ZAVODOV IN DRUŠTEV
ATTIVITÀ DEI NOSTRI ISTITUTI E DELLE NOSTRE SOCIETÀ
ACTIVITIES BY OUR INSTITUTIONS AND ASSOCIATIONS

Antonio Celona
NECTON MARINE RESEARCH SOCIETY

La Necton Marine Research Society è nata nel 2001 con lo scopo di creare ed attuare programmi di ricerca volti alla salvaguardia ed alla conservazione dell'ecosistema marino.

La società è formata da biologi marini, naturalisti e sommozzatori, estremamente preparati e di provata esperienza nel campo della biologia marina, provenienti da istituti di ricerca conosciuti in Italia e all'estero. I progetti vengono portati a termine con professionalità ampiamente riconosciuta ed apprezzata, ed i risultati dei progetti sono presentati in convegni e riviste scientifiche internazionali.

La Necton si occupa in modo particolare dello studio della biologia e dell'ecologia dei grandi pelagici, dei rettili marini e dei cetacei. La Necton aderisce all'AGCI pesca (Associazione Generale delle Cooperative Italiane), è consorziata con il Co.Si.Pe (Consorzio Siciliano Pesca) all'interno del quale si occupa dei problemi legati all'impatto degli attrezzi da pesca sulle specie marine protette. È inoltre partner della società cooperativa Biotecno per progetti di ricerca, collabora con la Banca Dati Italiana Squalo Bianco, il Fondo Siciliano per la Natura, il Mediterranean Shark Research Group, l'Istituto di ricerca Aquastudio, l'Associazione Asterisco e nel 2005 ha firmato una convenzione con l'Università di Messina, grazie alla quale gli studenti possono compilare le proprie tesi di laurea collaborando ai suoi programmi di ricerca. È registrata all'Anagrafe Nazionale delle Ricerche presso il Ministero dell'Università e della Ricerca Scientifica e Tecnologica. La Necton ha sede legale a Catania, ma è presente anche a Messina con una sede operativa, attrezzata di un piccolo laboratorio, ed opera in tutto il bacino del Mediterraneo.

La Necton si occupa in modo particolare di:

realizzazione di studi e ricerche sull'ambiente marino e sulle componenti biotiche e abiotiche, realizzazione e gestione di servizi tecnici inerenti alle attività legate al mare, valutazione delle risorse ittiche, ricerca applicata alle attività di pesca, attività di monitoraggio ambientale, valutazioni di impatto ambientale, indagini conoscitive, piani di gestione delle aree marine protette, ricerca applicata all'acquacoltura e alla maricoltura, attività di prelievo di organismi marini a fini scientifici o didattici (compresa la raccolta di organismi per acquari e parchi marini), realizzazione, gestione e promozione di iniziative di recupero ambientale, supporto tecnico/scientifico finalizzato allo studio e alla gestione di aree marine protette, formazione professionale su tematiche legate al mare, divulgazione scientifica, progetti di ecoturismo, organizzazione di campi natura e campi studio, realizzazione e promozione di iniziative di recupero e valorizzazione della cultura e delle tradizioni legate al mare, attività di editoria, progettazione e produzione di strumenti multimediali ed audiovisivi inerenti al mare.

Tra i progetti in atto citiamo: il Progetto Mobula che si occupa della biologia e dell'ecologia della manta, *Mobula mobular* nelle acque siciliane, il Progetto Delphis, che studia l'interazione tra cetacei e pesca ai cefalopodi nei mari Tirreno e Ionio, ed il Progetto Caretta, sulla marcatura e ricattura con sistemi non invasivi della tartaruga marina *Caretta caretta*. Tra i progetti ultimati o in fase di ultimazione ricordiamo il Progetto Xiphias, studio su biologia ed ecologia del pescespada, *Xiphias gladius* nelle acque dello Stretto di Messina, il Progetto SCS, che si occupa della biologia e dell'ecologia dei cetacei stanziali ed in transito nello Stretto di Messina, il Progetto pesca e tradizione, che riguarda l'impatto ecologico e socio economico della pesca tradizionale al tonno rosso, *Thunnus thynnus* nell'area dello Stretto di Messina, il Progetto Research on the Sea, che riguarda la diffusione dei risultati scientifici delle ricerche sui mammiferi marini e tartarughe all'interno delle scuole Nazionali (in collaborazione con Ass. Asterisco), ed il 1st Lampedusa Marine Mammals Workshop, un ciclo di corsi su biologia ed ecologia dei mammiferi marini per studenti universitari tenutosi nell'area dell'Arcipelago delle isole Pelagie.

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Martina Orlando Bonaca

MICROHABITAT PREFERENCES OF COASTAL COMBTOOTH BLENNIES FAUNA (BLENNIIDAE) IN THE GULF OF TRIESTE

Ph.D. Thesis
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The combtooth blennies (family Blenniidae) are benthic bottom-dwellers which occur in coastal waters. Nowhere else are these blennies as abundant and diverse as in the Mediterranean Sea, where researchers have found 19 species. Fifteen of them live in the Slovenian sea. Since they are without economic value and they inhabit hard bottoms where the collecting of samples with trawls is impossible, they were poorly known just thirty years ago. Knowledge about these benthic species has increased during the last decades by the use of non-destructive visual census methods, aided by SCUBA. However, the ecological factors affecting their distribution in the environment and their microhabitat preferences remain poorly understood.

Combtooth blennies exhibit male parental care, with territorial males preparing nests in the spring-summer period. They then invite females inside in order to lay eggs. After fertilization the males guard and defend the eggs against predators, until they hatch. Males can be distinguished from females in most species, because they exhibit distinctive colors during the breeding season. Many blennies utilize endolithic holes, which are bored by etching bivalves, like the date mussel *Lithophaga lithopaga* and the smallest *Gastrochaena dubia*. Bigger blennies species prepare their nests in cracks and crevices among boulders.

The aim of the study was to find out differences among blennies species in microhabitat preferences and in the utilization of different endolithic holes as nests during the breeding season. During the period 1998-2005, the blennioid assemblage was studied along the Slovenian coastal sea. The total number of surveys was 286, all conducted diurnally. The methods used were: vertical transects, horizontal transects, all-occurrence sampling, linear cinetransects and the square method. During diving the microhabitat variables of the site where the blenny was found were annotated, in order to understand the ecological demands of blennies during the breeding season. The importance of biotic (vegetation, benthic fauna, floral cover) and abiotic (depth distribution, illumination, bottom type) factors were supposed to be very important in the microhabitat choice. For endolithic species of blennies we searched for possible correlations between a single species and the endolithic hole's parameters (type of shell, width, length, position and inclination of the hole). These observations were carried out in the natural environment and during laboratory experiments, as well.

During the study period a total of 14 blennies species were recorded in the Slovenian coastal sea, with the use of the above mentioned non-destructive methods. All 14 species were detected using the all-occurrence sampling method, which showed the best results with the lowest sampling effort in terms of number of surveys. The presence of the cryptobenthic species *P. zvonimiri* was confirmed only with the all-occurrence sampling method. The highest number of species was recorded in the first meter of depth, where 10 species were present. The number of species then decreased toward deeper waters.

Four species of blennies showed themselves to be indiscriminate in their microhabitat choice, as they were found in 25 to 50% of the inspected microhabitats. These species include *P. incognitus, L. dalmatinus, A. sphynx* in *P. rouxi*. Ten species were classified as infrequent, as they were recorded in less then 25% of the microhabitats.

A Canonical Correspondence Analysis (CCA) was carried out in order to determine the distribution and codependence of 13 blennies species with 13 environmental variables. The results show that the structure of the blennies assemblage in the Slovenian sea is affected by a large number of interplaying factors. The bottom composition, depth, benthic flora and fauna, incorporating both biotic and abiotic variables, are some of the factors responsible for the distribution of coastal blennies. Species inhabiting shallow waters showed a high positive correlation with boulders bigger than 2 m, the presence of mussels and cirripeds, and the presence of empty holes excavated by the date mussel. Species inhabiting deeper waters showed a high correlation with precoralligenous formations, which are frequent on a hard bottom below 4 m depth.

During the surveys, 203 individuals from 10 blennies species were recorded in dwelling places, which are mostly used as nests. Small benthic fish are nesting in holes and crevices in order to defend themselves and the fertilized eggs from predators. Holes, which have narrower entrances than crevices, enable the territorial male that is inside to prevent the entry of other males, and thus to defend its hole. Species found in crevices or in places among boulders were only rarely recorded in nests. Species-specific differences in the utilization of holes were found for species that nest in endolithic holes. The results show that some species *(L. dalmatinus* and *A. sphynx)* choose holes that are little larger than their heads, which prevent small males from being dislodged by bigger ones. *L. dalmatinus* was recorded in date mussels' holes, in holes bored by *G. dubia*, and it was the only species also found in holes made by the yellow boring sponge *Clione celata*, which enable the smallest Adriatic blenny to avoid interspecific competition for the hole. Two bigger species (*P. rouxi* and *P. zvonimiri*), which are probably

less exposed to intraspecific competition for holes, also choose holes with an entrance diameter twice as big as their head diameter. *P. zvonimiri* also occupies holes much longer than its body, while the majority of smaller endolithic species dwell in holes that are approximately as long as their body. Species living in shallow waters prefer sunny hole positions, while *P. rouxi* and *P. zvonimiri* were mostly found in the shade of boulders and rocks. *A. sphynx*, which lives in the mediolittoral belt, avoids competition with other species by choosing vertical holes, while other species mostly occupy horizontal holes.

Six males of *P. zvonimiri* and 6 males of *P. incognitus* were used in laboratory experiments. During 6 series of experiments we found a positive correlation between the rank (the dominance) of males and the access to the hole. Our findings confirm the thesis of "wide territoriality", in which a male defends a net of holes and not just one hole in his territory. *P. zvonimiri* predominates over *P. incognitus*. In the natural environment *P. zvonimiri* dwells in a smaller number of microhabitats then *P. incognitus*. For this reason, it has to defend more successfully its own territory and nests than *P. incognitus*, which is a widely distributed species and could find nesting holes in very different microhabitats.

The results of single-species experiments confirm our field observations, in which *P. zvonimiri* and *P. incognitus* males choose the widest available holes. Both species dwell in holes that are longer than their bodies. In two-species experiments, males of *P. incognitus* were forced to occupy the shortest holes, which confirms the dominance of *P. zvonimiri*. During the series of experiments with differently inclined holes in the aquarium, the two dominant males mostly occupied horizontal holes and holes with 135ş of inclination.

The dissertation gives new knowledge on the ecology of blennies and also useful information about the ecological conditions of hard bottom microhabitats in the marine coastal area. These kinds of habitats are very important not only for blennies, but also for the whole fish assemblage and benthic flora and fauna.

Janja Francé

THE ECOLOGICAL CHARACTERISTICS OF PLANKTONIC DINOFLAGELLATES (Dinophyceae) IN THE GULF OF TRIESTE WITH AN EMPHASIS ON TOXIC SPECIES

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Dinoflagellates are important members of the phytoplankton community in the coastal sea. In temperate regions, dinoflagellates achieve maximal abundance in the late spring and summer period. They are generally well adapted to environments with low turbulence and low nutrient concentrations. Their directional swimming ability is one of adaptation strategies to overcome scarce nutrient availability. Diel vertical migrations through the water column allow dinoflagellates an adequate nutrient uptake, as well as to avoid grazing and high light intensities. The ability to actively choose their depth is an important contributing factor to dinoflagellate bloom formation, but differs among different species and under different environmental conditions. Dinoflagellates blooms may sometimes have harmful consequences on marine ecosystem and humans. Among various types of intoxication, two are of major importance in the Gulf of Trieste, as their causative organisms are commonly found in the northern Adriatic. First is diarrhetic shellfish poisoning (DSP), which is caused mainly by various species of the genus *Dinophysis*. Second, and more dangerous, is paralytic shellfish poisoning (PSP) caused by some species of the genus *Alexandrium*. At shellfish farms on the Slovenian coast, DSP occurs almost every year, and results in the ban on shellfish sale. PSP, however, has not been observed in this area thus far, despite the persistent occurrence of *Alexandrium* in water samples. Recently, there has been a growing interest in new toxin types, such as the yessotoxins. They are produced by *Lingulodinium polyedrum* and *Protoceratium reticulatum* regularly found in the phytoplankton community of the Gulf of Trieste.

The aim of the study was to advance the knowledge of dinoflagellate ecology in two ways. In the first place, long-term data were analyzed in order to uncover seasonal occurrence patterns of toxic dinoflagellates and to determine the predictability of succession of most recurrent *Dinophysis* species. Sea water was sampled from 1995 to 2003 and examined with the use of an inverted microscope. Data from different depths at two sampling sites in the vicinity of shellfish farms were compared. Correlations between some environmental parameters and the abundance of toxic species for the year 1997 were investigated as well. The occurrence pattern of most frequent and abundant *Dinophysis* species was analyzed using the STATIS multivariate analysis. Secondly, to asses the ability of the dinoflagellate species to perform active vertical movements, two 24-hour samplings of the water column were carried out. In order to include the entire set of environmental stresses that dictate the organisms' response, sampling in the natural water environment was chosen. Samples were taken at 4-hour intervals at different depths. The first 24-hour sampling was performed in November during a period of a mixed water column, while the second was performed under stratified water column conditions in June. Combining the findings from the two parts of this study, improvements were suggested

for more effective management and mitigation of harmful dinoflagellate blooms.

During the nine year investigation period, 20 species of toxic and potentially toxic dinoflagellates from the Gulf of Trieste were discovered. 16 *Dinophysis* species were present in the water column, predominantly in the second half of the year. They displayed two yearly abundance maxima in the surface layer: the first in late-spring and the second in autumn. In the middle layer, there was no spring peak, and cell abundance increased gradually from June until the autumn peak. Only four *Dinophysis* species were predominant in water samples and showed a clear succession through time: *D. sacculus* is a typical late-spring species that peaks in June. Simultaneously with *D. sacculus*, *D. caudata* appears in water samples, but reaches its maximum in September. *D. rotundata*, which is present in samples throughout the second half of the year, peaks twice, once in June and again in October. Finally, the presence of *D. fortii* is limited to the autumn period. The lowest abundances were observed (<25 cells l^{-1}) for *D. rotundata*, whereas the other three species occasionally attained cell numbers high enough to cause DSP events (from 100 to several 1000 cells l^{-1}).

Generally, the *Alexandrium* species were present in water samples through the whole year. They were most abundant during the spring and early summer period, while their lowest abundance was recorded between August and December. Small species were predominant in the *Alexandrium* genus, except in June, when *A. pseudogonyaulax* prevailed. The seasonal dynamics of *Alexandrium* was similar in the surface and middle water layers, though abundances were slightly lower in the latter. During peaks, the *Alexandrium* species reached abundances as high as several 100 to several 1000 cells l^{-1}. Two other potentially harmful species, *L. polyedrum* and *P. reticulatum*, whose appearance was limited to the late-spring and summer months, reached their maximum abundance in June.

Some statistically significant correlations were found between species abundance and environmental parameters in the surface layer at station 0024 in 1997. Salinity seemed to be the most important environmental factor, as a number of correlations were found between this factor and species abundance. Correlations between salinity and *Dinophysis* were species specific, while for the *Alexandrium* genus correlation was strictly negative in concordance with literature data. As regards cell numbers during peaks, all species of interest showed considerable inter-annual variability, although they had stable seasonal occurrence patterns. This possibly implies the involvement of stochastic processes, such as wind and current driven cell accumulation and specific environmental variations. Harmful outbursts of such organisms are thus not easily predictable in spite of our knowledge of seasonal dynamics.

The 24-hour samplings both under mixed water column conditions in November and stratified water column conditions in June showed the susceptibility of the shallow coastal sea to short-term alterations. Vertical profiles of temperature, salinity, and density in November confirmed the mixed conditions, since the parameters were uniform along the water column. They changed temporarily only within the first two meters due to the passage of a freshwater front. Despite uniform vertical conditions, migration of the dinoflagellate community through the water column was observed. Autotrophic dinoflagellates (the *Alexandrium* and the *Heterocapsa* species) displayed distinctive day-night dynamics: during daylight, cells accumulated in the surface layer, but gathered mostly near the bottom during the night. Species from the mainly heterotrophic genera *Protoperidinium* and *Diplopsalis* altered their vertical position as well, yet these movements did not coincide with the day-night cycle.

Water column conditions were even more variable during the June 24-hour sampling, when water column stratification was repeatedly disturbed and re-established. Vertical movement under such conditions differed substantially between species. Diel vertical migrations were observed for most of the predominating autotrophic dinoflagellates: *Heterocapsa* spp., *Prorocentrum micans*, *P. triestinum*, *Scrippsiella* spp., *Ceratium furca* and *D. sacculus*. By contrast, diel vertical migrations were not observed for the mostly heterotrophic naked dinoflagellates, for the *Protoperidinium* species and phagotrophic *D. rotundata*.

The expected difference between the vertical migrations of dinoflagellates under stratified and mixed water column conditions was therefore not confirmed. In a constantly changing shallow water column environment, dinoflagellates may preserve their movement pattern as a possible advantage in the exploitation of their environment as well as to avoid grazing. Diel vertical migrations are thus more likely an expression of internal rhythms than a response to environmental conditions. The great dissimilarity in the timing and degree of vertical movements among different species and the possible accumulation of cells in thin layers dictate careful planning of a monitoring programme for toxic species. Thus, sampling should be carried out within as many layers as possible. Integrated water samples from layers of the entire water column should be examined for easier, more accurate and more rapid evaluation.

Tinkara Tinta

THE INFLUENCE OF MARICULTURE ON PLANKTONIC BACTERIAL COMMUNITIES IN THE PELAGIC ZONE OF PIRAN BAY

B.Sc. Thesis
University of Ljubljana, Faculty of Chemistry and Chemical Technology, 2006
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The numerous documented negative effects of mariculture on the environment are connected especially to the accumulation of uneaten food and feces in sea water and in/on the sediment. To evaluate the influence of the fishery pollution on heterotrophic planktonic bacterial communities, spatial and temporal differences in the abundance, growth rates and bacterial community structure were studied in the Gulf of Trieste (Northern Adriatic) on 18 October 2005. At the same time, differences in the chemical parameters (total suspended solids (TSS) and nutrient levels) and physical parameters (basic oceanographic parameters) were followed together with 24-hour current measurements.

Sampling was carried out at the stations around the fish cage and along the transect from the cage located inside the Bay of Piran towards the reference station located 5,400 m away in the middle of the Gulf of Trieste. A new sampling system was designed to provide accurate data at different sampling locations. Underwater samplers were constructed in a circle at a distance of 8 m around the operating fish cage (6 locations) and another circle at a distance of 20 m around the fish cage (6 locations). Each bottle (5 l) was fixed on an underwater metal frame, 5 m below the surface. All sampling bottles were opened simultaneously collecting seawater samples at all location around the fish cage. Sampling was performed before feeding and 3 hours after the feeding of the fish. The result for each parameter was expressed as a ratio ($K= C_{bf}/C_{af}$) between the values before and after feeding.

Preliminary results showed temporal and spatial distribution of bacterial abundance and growth rates as well as TSS, ammonia and nitrate concentrations, which were related to distance from the fish farm and to pre-feeding and post-feeding conditions. In the vicinity of fish cage, the abundance value of heterotrophic bacteria was 2.5-times higher comparing with the bacterial abundance at the reference station. Significantly higher (3-times) was also the growth rate of the total bacterial population. High P/B ratio also reflects intensive bacterial metabolic process in the vicinity of the fishery.

Bacterial community compositions along the transect were determined with new molecular methods, such as FISH (*Fluorescent in situ Hybridization*) and DGGE (*Denaturing Gradient Gel Electrophoresis*). Analysis of the bacterial community compositions with FISH method has shown that bacterial groups from subclasses *α-*, *β-* and *γ-Proteobacteriae* as well as *Cytophaga-Flavobacteria-Bacteroides* group and *Vibrio* group were present in all the samples along the transect. Although all bacterial groups were present in the water samples along the transect, there was a great difference in percentage of the each group at separate stations. In the centre of the fish cage dominated the group of nitrifying ammonia-oxidizing bacteria, compared to remote stations on the transect, where *Cytophaga-Flavobacteria-Bacteroides* group and *Vibrio* group were predominant. Analysis of the bacterial community compositions with DGGE method has given similar results that also show the presence of various bacterial groups within a particular sample as well as spatial differences along the transect.

Significant decrease in bacterial carbon production was observed with distance from the pollution source according to the radioactive leucin method. The preliminary results of metabolically active bacterial groups, determined with the BrdU-DGGE method, have shown that the same bacterial groups were metabolically active at all stations along the transect.

Measurements of temporal differences in the abundance and growth rates of the total bacterial population were carried out at the stations around the fish cage before and three hours after the feeding of the fish. After the feeding, a significantly higher bacterial carbon production was measured. The results were comparable with the increased ratio between the total suspended solids (TSS) and nutrient levels measured before and after feeding. The increased bacterial carbon production could be due to the higher concentrations of the TSS and nutrient levels in the surrounding of the fishery.

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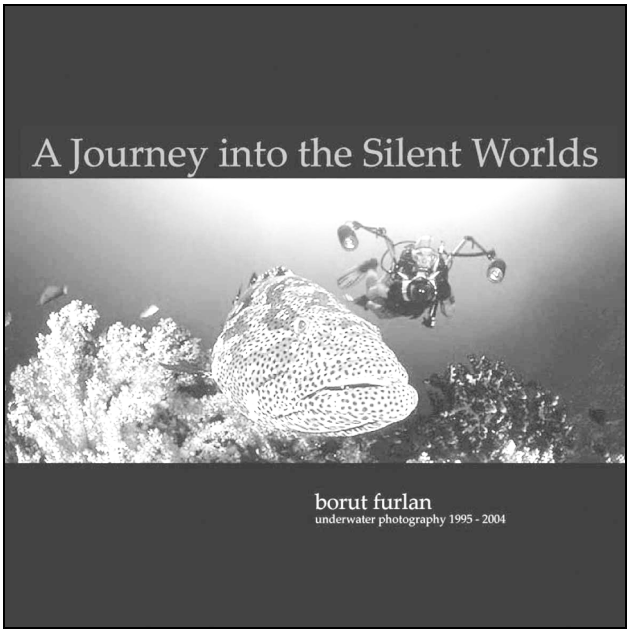
REVIEWS

Borut Furlan: A JOURNEY INTO THE SILENT WORLDS
Didakta d.o.o., Radovljica, 2006

The words written in the book *A Journey into the Silent World*s probably tells us more about its author, the underwater photographer Borut Furlan, than I can possibly utter in this short review. Namely, Borut says the following: "The underwater casing. Every single time I get hold of it, I am seized by irrepressible love towards it, by a very special respectful relationship, almost is if touching a relic, the Holy Grail."

It is only a man with this kind of attitude towards the equipment and everything that he does with it that can reach perfection in this technique. In underwater photography this of course holds true incomparably more than in land photography, considering that under water you cannot rectify any mistakes, change films, batteries, lenses and other trifling things that are, on the land, something completely obvious and the matter of plain routine. Apart from this, it can be a single inadvertence, a small foreign particle on the O-ring that seals the housing, which can not only spoil your dive but also destroy the camera and flash at the same time. The weight of the equipment and restricted air as well as all other technical details of diving are additional factors that often spoil the underwater photographer's joy.



Underwater photography thus demands a strict planning of everything that the photographer intends to shoot in a short, limited time. To make a top-notch photograph, to freeze an unrepeatable moment into eternity, to be in the right place at the right time, all this is difficult for any photographer, but even more so for the underwater photographer. Without professional attitude and extreme devotion to what you do, a top achievement is only a wish that will evade you on and on and at all times.

Borut is no doubt one of the very few non-professionals, but lives and works precisely in the way he would if he was a professional photographer. The supreme underwater photography, presented in his monograph – the first of this kind in Slovenia – is therefore not the fruit of fortuitousness, luck or merely numerous hours spent under the surface of the sea, but the result of a complete mastery of diving, the equipment in which he has been investing for not only years but decades, and of perfecting his own photography technique at the same time. It is the fruit of experience and knowledge, which through years gradually transformed into top-class photographs. All this would of course not have been possible without a trifle of luck and interlacement of circumstances, which enabled him to make many a perfect shot, although we could say that there are photographs in this book that have been made owing to the very lack of this same luck and interlacement of favourable circumstances. It is only work, self-sacrifice and Borut's devotion to the sea and its inhabitants that enable us to delight in leafing through the author's "Silent Worlds". In them, he takes us from the well known underwater landscapes of the Adriatic and the Mediterranean, through tropical Caribbean and Indo-Pacific corral reefs, to the cold and dark waters of the Norwegian fjords. But wherever we are, we witness some incredible and almost panoramic pictures of underwater landscapes, wrapped in the deep blueness of the sea, their inhabitants and incredible forms and patterns of various living beings, which the author conjures up with a considered composition and an exceptional feeling for the underwater setting.

The book is indubitably also a great achievement as far as its design is concerned. It may be interesting that the presented photographs were chosen, from a true multitude of them, by the book's designer and not its author. Although I believe that the selection by the author himself would have been probably different and possibly even better, it has to be admitted that with the selection of photographs, which are highly concordant in terms of colour and composition, a more than suitable objective has been achieved by her, the designer. The number of published photographs themselves, size, paper and the quality of print are also such as becoming for the book of this type. The only minor deficiency perhaps lies in the selection of colour and size of the letters, which could be larger, more contrasting and thus

easier readable for all of us who can no longer boast hawk eyes.

Borut Furlan's monograph The Silent Worlds was published by Didakta Publishing House, which deserves all the praise for its decision to tackle this undoubtedly highly demanding and, for the Slovene circumstances, unique project.

Plunge into The Silent Worlds and enjoy yourselves in them.

Tom Turk

Iztok Škornik: STO SLOVENSKIH PTIC
Založba Modrijan, Ljubljana, 2006, 263 str.

V začetku oktobra 2006 je v okviru založbe Modrijan izšla knjiga koprskega ornitologa Iztoka Škornika. Gre za lično oblikovano monografijo, ki nam predstavlja stotero portretov ptic, posnetih pretežno s tako imenovano digiskopsko tehniko. Pri tej tehniki fotografiramo ptice skozi teleskop za opazovanje ptic z digitalnim fotoaparatom. Prednosti tovrstne tehnike so velike, saj lahko pridemo hitro do velikega števila posnetkov, ne da bi pri tem motili ptice. Ni naključje, da je ravno Iztok Škornik prvi izdal takšno knjigo. V Sloveniji je bil namreč med prvimi ornitologi, ki so začeli fotografirati z digiskopsko tehniko, svoje izkušnje pa je redno opisoval na spletnih straneh v Sloveniji in Evropi ter veliko obetajočim fotografskim navdušencem v obliki tečajev. Po drugi strani pa je bil že čas, da svoje vedenje in izkušnje preoblikuje v pisano besedo. V knjigi tako opisuje sto slovenskih ptic iz 18 redov, med katerimi je velika večina močvirskih oz. vodnih ptic. Med njimi so tudi nekatere, ki se v Sloveniji pojavljajo zelo poredkoma (npr. plamenec in klavžar). Vsakega od teh redov opiše, temu sledi opis izbranih vrst iz posameznega reda. Besedilo je dovolj strokovno in tekoče berljivo. Ponuja splošni opis vrste in osnovne podatke o njeni biologiji. Avtor sam priznava, da ni želel narediti priročnika za ptice, ampak knjigo fotografij. Zasnovo knjige je torej zgradil na fotografskem gradivu, ki mu je dodal besedilo, in ne obratno, kakor običajno poteka nastajanje knjige. Treba je priznati, da so v knjigi res izjemne fotografije. Več kot polovico izvrstnih fotografij je avtor prispeval sam, druge pa 15 domačih in tujih ornitologov.

Knjiga je posebna z več vidikov. Gre za prvo tovrstno knjigo fotografij ptic, fotografije v njej so večinoma nastale z digiskopsko tehniko, nenazadnje pa je nenavaden tudi format.

V knjigi je avtor združil svoje ornitološko vedenje, fotografske veščine in oblikovni posluh. Delo je Škornikov knjižni prvenec, sicer pa ima avtor v svojem strokovnem opusu kar nekaj strokovnih del s področja ornitologije. Smiselno bi bilo pričakovati, da bo knjižni prvenec kmalu dobil bratce in sestrice.

Lovrenc Lipej